Applied Epidemiology in Victoria

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Originality Statement

I hereby declare that this submission is my own work and that to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at the Australian National University or any other educational institution, except where due acknowledgement is made in the text. Any contribution to the research by others is explicitly acknowledged in the thesis. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation or linguistic expression is acknowledged.

Sophia Bowman-Derrick

Date: 8 November 2019

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Thesis abstract

The Communicable Diseases, Epidemiology and Surveillance (CDES) branch, Victorian Government Department of Health and Human Services (DHHS), works closely with the Microbiological Diagnostic Unit Public Health Laboratory (MDU) to conduct surveillance of communicable diseases in Victoria. From February 2018 to December 2019, I attended field placements at both CDES and MDU. This thesis comprises projects which together meet the requirements for the Masters of Philosophy (Applied Epidemiology) (MAE). The projects include: an analysis to assess changes in the epidemiology of invasive pneumococcal disease in Victoria, 2008–2018; an evaluation of the surveillance of antimicrobial resistance in Victoria; a cross-sectional study of the genomic epidemiology of vancomycin-resistant enterococci, Victoria, November 2018; investigation of an outbreak of salmonellosis at a Mother's day lunch in regional Victoria, and recruitment of case-controls for a multi-jurisdictional outbreak investigation of hepatitis A. In addition, this thesis describes teaching activities undertaken as part of the MAE.

Acknowledgements

I would first like to acknowledge Dr Emma Field. Thank you for being an incredibly kind supervisor who was always available for discussion, and for your helpful feedback on earlier drafts. Associate Professor Stephen Lambert took over my supervision in the final months of the MAE. Thank you, Stephen, for your extremely useful comments in the final stage and for pushing me to improve my writing with each draft.

Thank you to Marion Easton and Professor Benjamin Howden for providing me with the opportunity to complete the MAE. Being immersed in CDES and MDU enabled me to learn an immense amount in a short space of time. I am grateful to all of the staff at both the DHHS and MDU for helping me to navigate the journey from veterinary clinician to epidemiologist in the human health field. I am particularly thankful to the MDU Epidemiology team — Mathilda Wilmot, Courtney Lane and Siobhan St George, for providing friendly support and advice.

Thank you to the MAE 2018 cohort for your friendship. Special thanks go to Ximena Tolosa (MAE 2017) and Laura Goddard (MAE 2019) for always being available to listen and discuss life, the universe and the MAE. Dave, thanks for being there through the highs and the lows, and for putting up with my ongoing list of ambitions. Lastly, thanks to Kevin for keeping it real and making me laugh on a daily basis.

Acronyms and Abbreviations

3GC	Third generation cephalosporins
23vPPV	23-valent pneumococcal polysaccharide vaccine
AEA	Australian Epidemiological Association
AGAR	Australian Group on Antimicrobial Resistance
AMR	Antimicrobial resistance
AMRL	Australian Mycobacterium Reference Laboratory
APAS	Australian Passive Antimicrobial Resistance Surveillance System
AST	Antimicrobial susceptibility testing
AURA	Antimicrobial Use and Resistance Australia
BSI	Bloodstream infection
CAR	Critical antimicrobial resistance
CARAlert	National Critical Antimicrobial Resistance Alert Surveillance System
CDES	Communicable Diseases, Epidemiology and Surveillance
CDPC	Communicable Diseases, Prevention and Control
CI	Confidence interval
CLSI	Clinical & Laboratory Standards Institute
CPE	Carbapenemase-producing Enterobacterales
CSF	Cerebrospinal fluid
DHHS	Department of Health and Human Services
EHO	Environmental Health Officer
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HAV	Hepatitis A virus
HCF	Healthcare facility
IPD	Invasive Pneumococcal Disease
IRR	Incidence rate ratio

LFF	Lesson from the field
LGA	Local government area
LIMS	Laboratory information management system
MAE	Masters of Philosophy in Applied Epidemiology
MDR	Multi-drug resistant
MDU	Microbiological Diagnostic Unit Public Health Laboratory
MIC	Minimum inhibitory concentration
MJOI	Multi-jurisdictional Outbreak Investigation
MLST	Multi-locus sequence type
MLVA	Multi-locus variable analysis of tandem repeats
NAAT	Nucleic acid amplification test
NEPSS	National Enteric Pathogen Surveillance system
NNDSS	National Notifiable Disease Notification System
NNN	National Neisseria Network
NSW	New South Wales
PCV7	7-valent pneumococcal conjugative vaccine
PCV13	13-valent pneumococcal conjugate vaccine
PDI	Peter Doherty Institute for Infection and Immunity
PHESS	Public Health Event Surveillance System
PHO	Public Health Officer
RR	Risk ratio
SNPs	Single nucleotide polymorphism
TB	Tuberculosis
VCRSU	Victorian Carbapenemase-producing Enterobacterales Surveillance and Response Unit
VHPSS	Victorian Hospital Pathogen Surveillance Scheme
VICNISS	Victorian Health-Care Acquired Infection Surveillance System

VPD	Vaccine preventable diseases
VRE	Vancomycin-resistant Enterococci
VREfm	Vancomycin-resistant *Enterococcus faecium*
WGS	Whole genome sequencing

Chapter I: Summary of Field Experience and Core Competencies

1. Masters of Applied Epidemiology (MAE) Experience

I attended two field placements concurrently throughout my MAE: the Microbiological Diagnostic Unit Public Health Laboratory (MDU) and the Communicable Diseases, Epidemiology and Surveillance (CDES) branch within the Victorian Government Department of Health and Human Services (DHHS).

1.1 Applying to the MAE

Until I commenced the MAE, I had been exclusively employed in small animal clinical veterinary practice, with the exclusion of a nine-month period conducting an honours project in the field of zoonoses and genomics at the Australian Animal Health Laboratory (AAHL). My experience at AAHL introduced me to the concept of public health and one health, which is how I came to be interested in completing further study in this field. It is only through speaking with other vets involved in public health that I came across the MAE programme, and decided to apply.

As someone who had not needed to use Microsoft Excel prior to 2018, the MAE has been an incredibly steep learning curve. The transition from clinical practice, where all of my problem solving was conducted in 15-minute appointments, to the longer-term work of public health research, was particularly challenging. However, through the MAE I have not only developed skills in public health research and analysis, I have also learnt about diseases I previously knew nothing about. Most importantly, the MAE has enabled me to develop a much clearer vision of how I can combine my skills as a veterinarian with public health work in the future.

1.2 CDES and MDU

The CDES branch works closely with MDU to conduct surveillance and coordinate the response to infectious diseases in Victoria. Epidemiologists within the CDES branch conduct surveillance of notifiable diseases, working closely with public health officers to manage outbreaks and other issues.

MDU sits within the Peter Doherty Institute for Infection and Immunity. MDU is Victoria's bacteriological reference laboratory, functioning in determining the public health significance of received samples, and providing guidance to health professionals and the Victorian DHHS on the response to infectious diseases. MDU is also the World Health Organization Regional Reference Laboratory for Invasive Bacterial-Vaccine Preventable Diseases and houses Doherty

Applied Microbial Genomics which researches the use of whole genome sequencing (WGS) in public health.

For my placement at CDES I was supervised by Marion Easton, the Principal Epidemiologist for antimicrobial resistance (AMR) and WGS. As Marion also works from MDU one day each week, this enabled her to contribute to supervision of all my MAE projects. At MDU I was supervised by the Director of MDU, Professor Benjamin Howden. I was also fortunate to receive mentoring and supervision from the MDU Epidemiology team.

1.3 Overall experience

Completing dual placements as part of my MAE came with both benefits and challenges. My understanding of the relationship between CDES and MDU was greatly enhanced by my role within both organisations. As the majority of my MAE projects focused on AMR, I found that a laboratory-based placement was essential to improve my understanding of antimicrobial susceptibility testing techniques and genomics.

In addition to the core MAE projects, within CDES I was involved in the following:

- Analysis of data for the Seasonal Influenza Survey 2018, which was conducted by the media department to assess knowledge of influenza among the Victorian population
- Attendance at weekly surveillance meetings
- Attendance at fortnightly WGS teleconferences held between MDU and CDES in order to coordinate the response to findings of WGS combined with the results of epidemiological investigations
- Conducting *Salmonella* food trawler questionnaires as part of cluster investigations (April and November, 2018)

My placement at MDU provided me with the following additional opportunities:

- From 2019, I was responsible for entering data for isolates that are eligible for inclusion in the National Alert System for Critical Antimicrobial Resistances. This enabled me to better understand the surveillance of AMR
- Attendance at the University of Melbourne Research Platform Services courses, including an introductory course for R Studio
- Attendance at seminars held at the Doherty, which covered topics including Indigenous health, hepatitis, vaccine preventable diseases, AMR and genomics

- An improved understanding of the surveillance and response of carbapenemase-producing Enterobacterales, which is coordinated by CDES and MDU
- An improved understanding of AMR surveillance systems, including the Victorian Hospital Pathogen Surveillance Scheme (VHPSS), which is coordinated within MDU

1.4 Summary of core activities related to course requirements

The core requirements of the MAE programme, as satisfied by each chapter within this thesis, are summarised in Table 1.

Table 1. Chapters satisfying MAE core competencies

	Core competency								
Chapter	Literature review	Data analysis	Outbreak investigation	Conduct an epidemiological study	Evaluate or design a surveillance system	Teaching activities	Communication to a lay audience	Late draft of an article for publication	Conference presentation
Chapter II: Changes in the Epidemiology of Invasive Pneumococcal Disease, Victoria, 2008–2018	✓	✓						✓	✓
Chapter III: Surveillance of Antimicrobial Resistance in Victoria	✓	✓			✓				
Chapter IV: A cross-sectional study of the genomic epidemiology of vancomycin-resistant enterococci, Victoria, 2018	✓	✓		✓			✓		✓
Chapter V: Salmonellosis at a Mothers' Day Lunch in Regional Victoria	✓	✓	✓						✓
Chapter VI: Recruiting Case Controls for a Multi-Jurisdictional Outbreak Investigation of Hepatitis A	✓		✓						✓
Chapter VII: Teaching Experience						✓			

Using data from the VHPSS, based at MDU, I conducted a ten-year review of invasive pneumococcal disease (IPD) in Victoria (Chapter II). I assessed changes in serotypes and antimicrobial susceptibility that occurred after the introduction of the 13-valent pneumococcal conjugate vaccine (PCV13) in Victoria. This analysis found that the majority of vaccine serotypes have reduced significantly after the introduction of the PCV13 in Victoria, and that levels of resistance to antimicrobials used for empirical treatment of IPD have not increased.

I evaluated existing surveillance activities for AMR in Victoria (Chapter III). This enabled me to understand the complexities of AMR surveillance, as well as providing me with experience conducting stakeholder interviews. The findings of this work will inform the development of the AMR surveillance and response unit within CDES.

I conducted an epidemiological study based at MDU (Chapter IV). This was a cross-sectional study of the genomic epidemiology of vancomycin-resistant enterococci (VRE) in Victoria. This experience enabled me to learn about the role of genomics in AMR surveillance, as well as the resources involved in conducting snapshot-style surveillance. The study built on knowledge of the burden of disease due to VRE in Victoria, and demonstrated the use of WGS for surveillance.

Chapter V and VI describe my experiences of outbreak investigation conducted within CDES. In chapter V, I describe an outbreak of salmonellosis in regional Victoria. Completing this early on in the MAE, I was able to apply learnings from MAE coursework to a real-world scenario. Chapter VI describes my experiences recruiting case-controls for a multi-jurisdictional outbreak investigation of hepatitis A. This experience provided me with an understanding of the resources involved in conducting case-control studies, as well as enabling me to gain additional experience in conducting case interviews.

Chapter VII summarises teaching activities that were completed during the MAE.

Chapter II: Changes in the Epidemiology of Invasive Pneumococcal Disease following the Introduction of the 13-valent Pneumococcal Conjugate Vaccine in Victoria

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1. Preface

Janet Strachan, the Principal Epidemiologist for Vaccine Preventable Diseases (VPD) at the Victorian Government Department of Health and Human Services (DHHS), initiated this project. The Victorian Hospital Pathogen Surveillance Scheme (VHPSS) is a voluntary laboratory-based system, which collects data on the serotypes and antimicrobial resistance (AMR) of all invasive (blood and cerebrospinal fluid) bacterial and fungal specimens in Victoria. The VHPSS is coordinated by MDU Epidemiology. VHPSS data are not regularly provided to the DHHS and there are currently no regular reports published, however data requests can be submitted to the VHPSS on an ad hoc basis.

The DHHS receives all notifications for cases of invasive pneumococcal disease (IPD), including serotype results generated by MDU. Currently, MDU does not conduct routine antimicrobial susceptibility testing for IPD isolates. The DHHS therefore does not receive AMR data for IPD except when diagnostic laboratories submit this information with notifications. The aim of this study was to determine firstly how serotypes of invasive *Streptococcus pneumoniae* isolates have changed in Victoria after the introduction of 13-valent pneumococcal-conjugate vaccine (PCV13) in July 2011. Secondly, we aimed to assess if the proportion of isolates with AMR changed with the introduction of PCV13. These findings would also inform the Communicable Diseases Epidemiology and Surveillance team at DHHS about the level of AMR in IPD isolates, and how frequently data requests should be lodged with the VHPSS as part of future surveillance activities.

1.1 My role

I was the primary investigator for this project, with guidance from academic and field supervisors, and the DHHS Epidemiologist for VPD. My role included the development of a research proposal, completion of an ethics application, performing data analysis in Stata, and writing up the study findings for submission to a peer-reviewed journal. I also presented findings from this project as a poster at the Australian Epidemiological Association (AEA) Conference, Brisbane, October 2019 and in oral format at the Communicable Diseases Control Conference, Canberra, November 2019.

1.2 Lessons learnt

This project taught me some of the challenges of working with real-world surveillance data including the need for extensive data cleaning, assessing the dataset for duplicates and evaluating missing data variables. The learnings from this project also informed my evaluation

of AMR surveillance in Victoria (Chapter III). I learnt that while there are valuable AMR data collected by the VHPSS, the amount of missing data makes it difficult to form strong conclusions. Laboratories do not consistently provide minimum inhibitory concentrations (MICs), or information on the antimicrobial susceptibility testing (AST) techniques and guidelines used to interpret AST. Consequently, this analysis relied on qualitative interpretations provided by the laboratories (susceptible, intermediate susceptibility, and resistant).

1.3 Public health impact

This analysis found that there were significant reductions in PCV13 serotypes after the introduction of the PCV13, however there were increases in non-vaccine serotypes. In addition, serotypes 3 and 19F increased after the PCV13 was introduced, despite their inclusion in the vaccine. Further investigation is required to determine if the increase in serotype 19F resulted from vaccine failures. While earlier evidence suggests that PCV13 is effective at preventing disease associated with serotype 3, the increase in the incidence of this serotype may result from the PCV13 being ineffective in the prevention of nasopharyngeal colonisation, resulting in a lack of herd immunity for this serotype. Local vaccine effectiveness studies are required to understand the increase in serotype 3 cases in Victoria. Implementing the use of PCV13 in adults may reduce the incidence of serotype 3, however this requires evaluation.

While there was no increase in levels of AMR identified by this analysis, only penicillin and third generation cephalosporins were able to be examined. Limitations identified in the AMR dataset informed recommendations made as part of the surveillance evaluation for AMR in Victoria (Chapter III). Minor modifications to the VHPSS would enable a more useful dataset which could be used for state-wide AMR surveillance. Specifically, the addition of data variables including AST technique and clinical guidelines used by the submitting diagnostic laboratory (to interpret MICs as susceptible, intermediate or resistant) would improve the data quality for AMR surveillance. Working with diagnostic laboratories to increase the proportion of submitted MIC results would also enhance the usefulness of the VHPSS for AMR surveillance of IPD and other organisms.

1.4 Acknowledgements

I acknowledge the following people for their assistance with this project:

- Janet Strachan and Marion Easton for initiating this project, and assisting with the study design
- Dr Emma Field for providing useful feedback on early drafts and for encouraging me to present this work at conferences

- Associate Professor Stephen Lambert for providing useful feedback on late drafts
- The coordinators of the VHPSS for enabling me to use this dataset

2. Abstract

Background: The 13-valent pneumococcal conjugate vaccine (PCV13) replaced the 7-valent pneumococcal conjugate vaccine (PCV7) on the childhood National Immunisation Program in July 2011. We assessed changes in pneumococcal serotypes and AMR profiles for IPD in Victoria in the period following PCV13 introduction.

Methods: IPD data for 01 January 2008 to 30 June 2018 were extracted from the Victorian Hospital Pathogen Surveillance Scheme, a voluntary laboratory-based system for invasive isolates. Cases were divided into three equal periods (pre-PCV13: 01 January 2008–30 June 2011; transition: 01 July 2011–31 December 2014; post-PCV13: 01 January 2015–30 June 2018). Serotypes were classified as those included in the PCV7 vaccine (PCV7), PCV13 but not the PCV7 (PCV13), and those included in neither vaccine (non-PCV). Antimicrobial susceptibility data were assessed using clinical breakpoints as interpreted by diagnostic laboratories. Using Poisson regression the incidence of serotypes was compared between time periods. Differences in the proportion of isolates with AMR to penicillin and third-generation cephalosporins (3GC) were assessed using chi-squared or Fisher's exact tests as appropriate.

Results: There were 3,865 IPD cases with available serotype data. The pre-PCV13 period included 1,215 cases; of which 99% had penicillin susceptibilities and 95% had 3GC susceptibilities. The post-PCV13 period included 1,277 cases; of which 100% had penicillin susceptibilities and 91% had 3GC susceptibilities. Univariate regression found that overall, the incidence rate ratio (IRR) for PCV13 serotypes decreased (IRR 0.54, 95% CI: 0.48–0.60) after the PCV13 was introduced. This was largely driven by reductions in serotype 19A (IRR 0.07, 95% CI: 0.02–0.30), 6A (IRR 0.04, 95% CI: 0.01–0.27), and serotype 1 (IRR 0.07, 95% CI: 0.02–0.30). Against this trend, serotype 3 increased (IRR 1.56, 95% CI: 1.24–1.95). Overall, the incidence of PCV7 serotypes decreased (IRR 0.63, 95% CI: 0.52–0.79), however serotype 19F increased (IRR 2.18, 95% CI: 1.49–3.17) after the introduction of the PCV13. The proportion of isolates resistant and non-susceptible to penicillin were comparable between the pre-PCV13 and post-PCV13 periods, however there were minor changes in the proportion of isolates susceptible to penicillin (increased from 88.8% to 91.6%, X^2=5.55, p=0.02). Antimicrobial susceptibility to 3GC in the pre-PCV13 and post-PCV13 periods were comparable.

Conclusion: While there were overall decreases in the incidence of serotypes included in the PCV13, serotypes 19F and 3 increased. Further investigation is required to determine the mechanisms underlying the increases of these serotypes. Levels of AMR did not increase however ongoing surveillance is essential to identify emerging changes.

Changes in Epidemiology of Invasive Pneumococcal Disease with the Introduction of the 13-valent Pneumococcal Conjugate Vaccine, Victoria, 2008–2018

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Introduction

Invasive pneumococcal disease (IPD), caused by *Streptococcus pneumoniae,* has clinical manifestations which include bacteraemia, meningitis, and sepsis (1, 2). IPD is associated with high levels of morbidity and mortality, with more than 800,000 deaths in children estimated to occur annually worldwide (3). Infants and those aged older than 60 years have the greatest risk of contracting IPD (2).

In Victoria, IPD has been notifiable since 2001 and all invasive *S. pneumoniae* isolates undergo capsular serotyping, performed by the Microbiological Diagnostic Unit Public Health Laboratory (MDU). Serotypes of IPD isolates are recorded in the Victorian Hospital Pathogen Surveillance Scheme (VHPSS), a voluntary laboratory-based surveillance system which includes organisms isolated from blood and cerebrospinal fluid (CSF), coordinated within MDU.

Since the introduction of vaccination for pneumococcal disease in Australia, there have been several changes to immunisation recommendations. A 23-valent pneumococcal polysaccharide vaccine (23vPPV) was funded for Victorians aged 65 years and older in 1998, and introduced under the National Immunisation Program in 2005 (4). In Australia, infants are vaccinated against IPD at two, four and six months (3 + 0), with a booster at 12 months for those with underlying medical conditions placing them at increased risk of IPD (3 + 1) (5). A 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in 2001, and included in the publicly funded childhood immunisation schedule in 2005 (6). The 13-valent pneumococcal conjugate vaccine (PCV13) replaced the PCV7 in the routine immunisation schedule in July 2011 (5). In Victoria, from July 2018, the vaccine schedule for children under the National Immunisation Programme will change to a 2 + 1 schedule, with vaccines at 2, 4 and 12 months.

The use of conjugate vaccines in children results in decreased levels of nasopharyngeal colonisation and therefore decreased transmission to adults for vaccine serotypes (7). The implementation of pneumococcal conjugate vaccine programs in children are therefore expected to result in herd immunity effects, with changes in all age groups (8). The 23vPPV contains 10 additional serotypes compared to the PCV13, however generates an inferior

immune response for most serotypes and the impact IPD in the over 65 year age group is debated (4, 9). Importantly, 23vPPV is not thought to effectively prevent vaccine-serotype colonisation (4).

Introduction of a new pneumococcal vaccine is associated with a process known as serotype replacement, whereby the prevalence of serotypes present in the vaccine decreases and that of serotypes not included in the vaccine increases (10, 11). Some serotypes of *S.pneumoniae* have developed resistance to commonly used antimicrobials which can make empirical treatment ineffective (2, 12). As the distribution of serotypes changes with the implementation of new vaccines, it follows that levels of antimicrobial resistance (AMR) may also change. For example, after the introduction of PCV7 in Victoria in 2005, serotype 19A, which is associated with multi-drug resistance, increased, impacting overall levels of AMR within IPD (5, 13, 14).

With this work we describe the epidemiology of IPD in Victoria from 2008 to 2018 and assess changes in pneumococcal serotypes and AMR profiles in the period following the introduction of PCV13.

Methods

Study sample

Data for all cases of IPD were extracted from the VHPSS with specimen collection dates from 01 January 2008 to 30 June 2018, inclusive. Duplicate isolates were defined as multiple specimen types or multiple isolates (of the same serotype) collected from a patient within a 30-day period. Where patients had both a CSF and blood isolate collected within 30 days of each other, only the CSF isolate was included.

Serotypes were grouped as PCV7 (those in the PCV7 vaccine only), PCV13 (those in the PCV13 but not the PCV7) and non-PCV (serotypes not included in either conjugate vaccine). Three equal time periods were allocated based on the date of specimen collection: pre-PCV13 (01 January 2008–30 June 2011), a transition period, to allow time for PCV13 to have an impact (01 July 2011–31 December 2014), and post-PCV13 (01 January 2015–30 June 2018).

Statistical analysis

Statistical analysis was conducted using Stata 15.1 (StataCorp, Texas, USA). Poisson regression was used to assess changes in the incidence of serotypes over time, including obtaining standard errors for parameter estimates (15).

Complete case analysis was conducted for isolates with antimicrobial susceptibility data. Cefotaxime and ceftriaxone antimicrobial susceptibility results were combined and reported as third generation cephalosporins (3GC). Where ceftriaxone and cefotaxime AST were discordant,

the higher minimum inhibitory concentration (MIC) was included in the analysis. AST data were analysed at two levels; using available clinical breakpoint data as interpreted by diagnostic laboratories (susceptible, intermediate or resistant), and using reported MIC values. Due to the extent of missing MIC data, isolates were further categorised as raised MIC (penicillin >0.06 mg/L; 3GC ≥0.5 mg/L). Differences in the proportion of susceptible, intermediate and resistant isolates were assessed using a chi-squared or Fisher's exact test. Changes in isolates with raised MICs over time were assessed using Poisson regression.

Ethical approval

This analysis was conducted under ethics approval from the Australian National University Human Research Ethics Committee, approval number 2018/690.

Results

There were 3,897 isolates reported to the VHPSS, excluding duplicates, from 01 January 2008–30 June 2018. An additional 32 isolates were excluded from this analysis as serotyping results were unknown (15 isolates not forwarded to MDU, 17 not viable for serotyping). This left a total of 3,865 isolates for analysis (3,767 blood, 98 CSF), representing 93% of Victorian notifications to the National Notifiable Diseases Surveillance System for the same period (16-18).

During the study period, the mean annual incidence of IPD was 6.5 cases per 100,000 population per year, ranging from 5.3 to 7.2 (Figure 1). The highest incidence was among those aged younger than 5 and 65 years and older. In contrast, among those aged 5 to 64 years, the incidence remained relatively stable. Over the study period, there were isolates from 1,727 (44.6%) females and 2,138 (55.3%) males. The rate in males was higher than that in females in all years for those aged 65 years and older, in all years except 2014 for those aged younger than 5 years, and in all years except 2008 and 2009 for those aged 5 to 64 years.

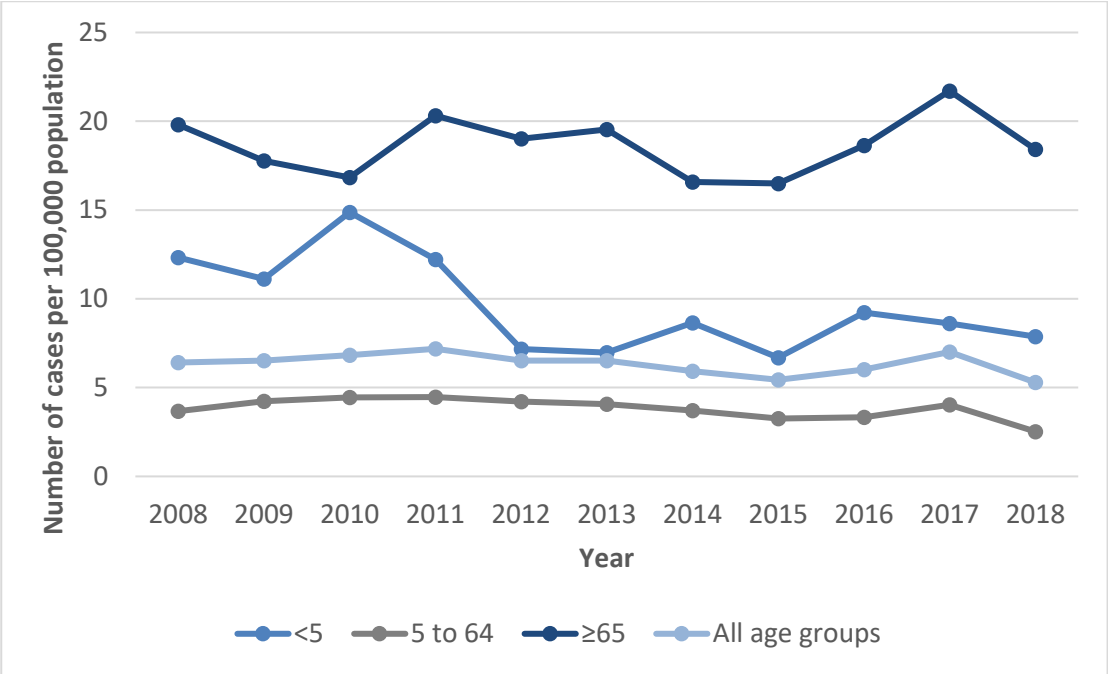

Figure 1. Incidence of IPD by age group in Victoria, as per isolates reported to the Victorian Hospital Pathogen Surveillance Scheme, 01 January 2008 to 30 June 2018[1]

Over the study period, the most prevalent serotypes were 19A (13.9%), 3 (10.6%), 7F (9.3%), 22F (8.5%), 6C (5.4%), and 19F (4.6%) (Table 1). Of all isolates, 53.9% were non-PCV. PCV7 isolates accounted for only 10.6% of serotypes, while 35.6% of isolates were PCV13 serotypes. Among children aged younger than 5 years of age, serotype 19A predominated (31.0%). Among those aged 5 to 64 years, serotype 7F was most prevalent (14.8%), followed by serotype 19A (12.1%). Among those aged 65 years and older, serotype 19A (12.0%) and serotype 3 (11.7%) were most prevalent.

[1] While the graph appears to display a decrease in incidence in 2018, this is due to seasonality as notifications of IPD tend to increase in quarter 3 (July to September)

Table 1. Numbers and proportions of serotypes by age group, 01 January 2008 to 30 June 2018. Data source: Isolates reported to the Victorian Hospital Pathogen Surveillance Scheme.

Percent prevalence of each serotype				
Serotype[2]	**<5 years n (%)**	**5 to 64 years n (%)**	**≥65 years n (%)**	**All ages n (%)**
PCV7 serotypes				
Serotype 9V	0	30 (1.6%)	15 (0.9%)	45 (1.2%)
Serotype 23F	0	8 (0.4%)	16 (1.0%)	24 (0.6%)
Serotype 18C	0	11 (0.6%)	5 (0.3%)	16 (0.4%)
Serotype 14	0	40 (2.2%)	18 (1.1%)	58 (1.5%)
Serotype 6B	1 (0.3%)	10 (0.5%)	20 (1.2%)	31 (0.8%)
Serotype 4	1 (0.3%)	41 (2.2%)	16 (1.0%)	58 (1.5%)
Serotype 19F	29 (7.8%)	87 (4.7%)	61 (3.7%)	177 (4.6%)
Total PCV7	31 (8.3%)	227 (12.2%)	151 (9.2%)	409 (10.6%)
PCV13 serotypes				
Serotype 5	0	1 (0.1%)	0	1
Serotype 6A	1 (0.3%)	16 (0.9%)	15 (0.9%)	32 (0.8%)
Serotype 1	2 (0.5%)	29 (1.6%)	2 (0.1%)	33 (0.9%)
Serotype 7F	18 (4.8%)	275 (14.8%)	67 (4.1%)	360 (9.3%)
Serotype 3	27 (7.2%)	193 (10.4%)	191 (11.7%)	411 (10.6%)
Serotype 19A	116 (31.0%)	225 (12.1%)	196 (12.0%)	537 (13.9%)
Total PCV13	164 (44.0%)	739 (39.9%)	471 (28.8%)	1374 (35.6%)
Non PCV[1]				
Serotype 22F	19 (5.1%)	154 (8.3%)	157 (9.6%)	330 (8.5%)
Serotype 6C	12 (3.2%)	58 (3.1%)	138 (8.4%)	208 (5.4%)
Serotype 9N	10 (2.7%)	94 (5.1%)	64 (3.9%)	168 (4.3%)
Serotype 33F	14 (3.8%)	72 (3.9%)	49 (3.0%)	135 (3.5%)
Serotype 23A	4 (1.1%)	48 (2.6%)	74 (4.5%)	126 (3.3%)
Serotype 23B	21 (5.6%)	37 (2.0%)	57 (3.5%)	115 (3.0%)
Serotype 15A	7 (1.9%)	29 (1.6%)	67 (4.1%)	103 (2.7%)
Serotype 35B	8 (2.1%)	35 (1.9%)	58 (3.5%)	101 (2.6%)
Serotype 11A	8 (2.1%)	39 (2.1%)	48 (2.9%)	95 (2.5%)
Serotype 16F	3 (0.8%)	35 (1.9%)	45 (2.7%)	83 (2.1%)
Serotype 8	2 (0.5%)	54 (2.9%)	15 (0.9%)	71 (1.8%)
Serotype 10A	13 (3.5%)	29 (1.6%)	20 (1.2%)	62 (1.6%)
Serotype 38	8 (2.1%)	12 (0.6%)	38 (2.3%)	58 (1.5%)
Serotype 15B	9 (2.4%)	16 (0.9%)	31 (1.9%)	56 (1.4%)
Serotype 15C	15 (4.0%)	17 (0.9%)	16 (1.0%)	48 (1.2%)
Other[3]	25 (6.7%)	159 (8.6%)	139 (8.7%)	323 (8.4%)
Total Non-PCV	178 (47.7%)	888 (47.9%)	1016 (62.0%)	2082 (53.9%)
Total all serotypes	373	1,854	1,638	3,865

[2] Serotypes listed including those which make up ≥2% of total for either the <5, 5 to 64 or ≥65 year age group

[3] Other serotypes include Serotypes 2, 6D, 7, 7B, 7C, 9A, 9L, 10B, 10F, 12F, 13, 15F, 17F, 18A, 18B, 20, 21, 22A, 23, 24, 24F, 29, 31, 34, 35A, 35F, 37, non-typeable

After the introduction of the PCV13 vaccine, the overall incidence of PCV7 (IRR 0.63, 95% CI: 0.52–0.79) and PCV13 (IRR 0.54, 95% CI: 0.48–0.60) serotypes decreased (Table 2, Figure 2). Notably, serotype 19F (IRR 2.18**,** 95% CI: 1.49–3.17) and serotype 3 (IRR 1.56, 95% CI: 1.24–1.95) increased, despite their inclusion in the PCV13. The decrease in PCV13 serotypes was due to reductions in the incidence of serotypes 1 (IRR 0.07, 95% CI: 0.02–0.30), 6A (IRR 0.04, 95% CI: 0.01–0.27) and 19A (IRR 0.23, 95% CI: 0.18–0.30).

Non-PCV serotypes increased (IRR 1.63, 95% CI: 1.51–1.76) after the introduction of the PCV13. The largest increases in non-PCV serotypes were 9N (5.07, 95% CI: 3.12–8.21), 15C (IRR 3.55, 95% CIL 1.45–8.68), and 15A (IRR 3.16, 95% CI: 1.67–5.99). Several serotypes demonstrated a change in IRR during the transition period in addition to the post-PCV13 period.

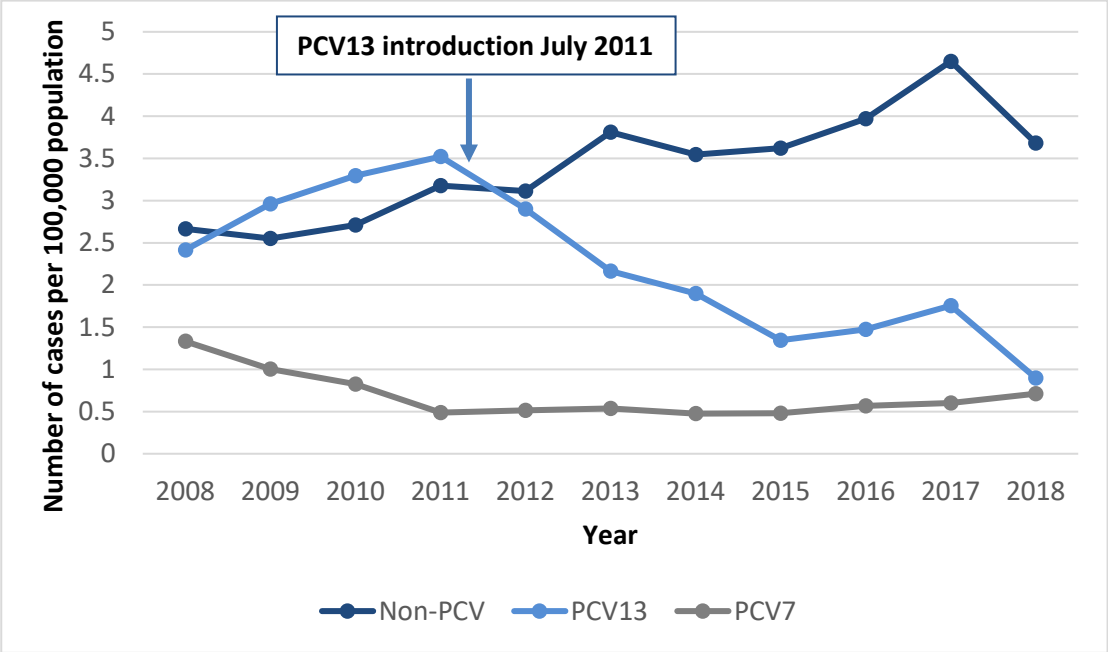

Figure 2. Number of cases of IPD, per 100,000 population, 01 January 2008 to 30 June 2018. Data source: Isolates submitted to the Victorian Hospital Pathogen Surveillance Scheme[4]

[4] While the graph appears to display a decrease in incidence in 2018, this is due to seasonality, as the number of notifications of IPD tends to increase in quarter 3 (July to September)

Table 2. Comparison of IPD serotypes by time period. Incidence rate ratio (IRR) as determined by Poisson regression, indicates the change in incidence compared to the pre-PCV13 time period as the baseline. Data source: isolates submitted to the Victorian Hospital Pathogen Surveillance Scheme.[5]

	Pre-PCV13		Transition			Post-PCV13		
	01 January 2008–30 June 2011		1 July 2011–30 December 2014			01 January 2015–30 June 2018		
	No. Cases	Mean cases/year	No. cases	Mean cases/year	IRR (95% CI)	No. cases	Mean cases/year	IRR (95% CI)
PCV7								
Serotype 14	26	7.4	18	5.1	0.63 (0.35–1.14)	14	4.0	**0.50 (0.26–0.95)**
Serotype 18C	14	4	1	0.3	**0.06 (0.01–0.50)**	1	0.3	**0.07 (0.01–0.50)**
Serotype 19F	37	10.6	53	15.1	1.3 (0.86–1.97)	87	24.9	**2.18 (1.49–3.17)**
Serotype 23F	15	4.3	5	1.4	**0.30 (0.11–0.83)**	4	1.1	**0.25 (0.08–0.74)**
Serotype 4	42	12	9	2.6	**0.20 (0.10–0.40)**	7	2.0	**0.15 (0.07–0.34)**
Serotype 6B	21	6	4	1.1	**0.17 (0.06–0.50)**	6	1.7	**0.26 (0.11–0.65)**
Serotype 9V	28	8	11	3.1	**0.36 (0.18–0.71)**	6	1.7	**0.20 (0.08–0.48)**
Total	183	52.3	101	28.9	**0.50 (0.40–0.64)**	125	35.7	**0.63 (0.52–0.79)**
PCV13								
Serotype 1	26	7.4	5	1.4	**0.17 (0.07–0.45)**	2	0.6	**0.07 (0.02–0.30)**
Serotype 19A	276	78.9	191	54.6	**0.63 (0.53–0.74)**	70	20.0	**0.23 (0.18–0.30)**
Serotype 3	107	30.6	124	35.4	1.05 (0.82–1.35)	180	51.4	**1.56 (1.24–1.95)**
Serotype 5	0	0	1	0.3	-	0	0	-
Serotype 6A	25	7.1	6	1.7	**0.22 (0.09–0.53)**	1	0.3	**0.04 (0.01–0.27)**
Serotype 7F	101	28.9	200	57.1	**1.80 (1.44–2.26)**	59	16.9	**0.54 (0.40–0.74)**
Total	535	152.9	527	150.6	**0.90 (0.81–0.98)**	312	89.1	**0.54 (0.48–0.60)**

[5] Statistical significance is defined as $p<0.05$, values meeting statistical significance are bolded: those demonstrating an increase are shown in red; those demonstrating a decrease are shown in green.

Non-PCV								
Serotype 11A	23	6.6	36	10.3	1.42 (0.85–2.39)	36	10.3	1.45 (0.86–2.43)
Serotype 15A	12	3.4	50	14.3	**3.79 (2.03–7.08)**	41	11.7	**3.16 (1.67–5.99)**
Serotype 16F	19	5.4	21	6.0	1.00 (0.54–1.86)	43	12.3	**2.09 (1.23–3.57)**
Serotype 22F	106	30.3	122	34.9	1.05 (0.82–1.34)	102	29.1	0.89 (0.69–1.16)
Serotype 23A	30	8.6	25	7.1	0.76 (0.45–1.28)	71	20.3	**2.19 (1.44–3.33)**
Serotype 23B	19	5.4	41	11.7	**1.96 (1.14–3.36)**	55	15.7	**2.68 (1.60–4.49)**
Serotype 33F	40	11.4	36	10.3	0.82 (0.52–1.27)	59	16.9	1.36 (0.92–2.02)
Serotype 35B	30	8.6	30	8.6	0.91 (0.55–1.50)	41	11.7	1.26 (0.79–2.01)
Serotype 6C	64	18.3	91	26.0	1.30 (0.95–1.76)	53	15.1	0.77 (0.54–1.09)
Serotype 9N	19	5.4	45	12.9	**2.15 (1.27–3.66)**	104	29.7	**5.07 (3.12–8.21)**
Serotype 10A	20	5.7	20	5.7	0.91 (0.49–1.68)	22	6.3	1.02 (0.56–1.86)
Serotype 8	24	6.9	21	6	0.80 (0.44–1.42)	26	7.4	1.0 (0.58–1.74)
Serotype 38	14	4	21	6	1.36 (0.70–2.67)	23	6.6	1.52 (0.79–2.94)
Serotype 15C	6	1.7	19	5.4	**2.88 (1.15–7.18)**	23	6.6	**3.55 (1.45–8.68)**
Serotype 15B	14	4	19	5.4	1.23 (0.62–2.45)	23	6.6	1.52 (0.79–2.94)
Others	57	16.3	112	32	**1.79 (1.31–2.43)**	154	44	**2.50 (1.86–3.35)**
Total	497	142.0	709	202.6	**1.30 (1.19–1.41)**	876	250.3	**1.63 (1.51–1.76)**

The average annual incidence of PCV13 serotypes reduced in all age groups after the introduction of the PCV13 (Figure 3). In contrast, the average annual incidence of non-PCV serotypes have increased in all age groups after the introduction of the PCV13.

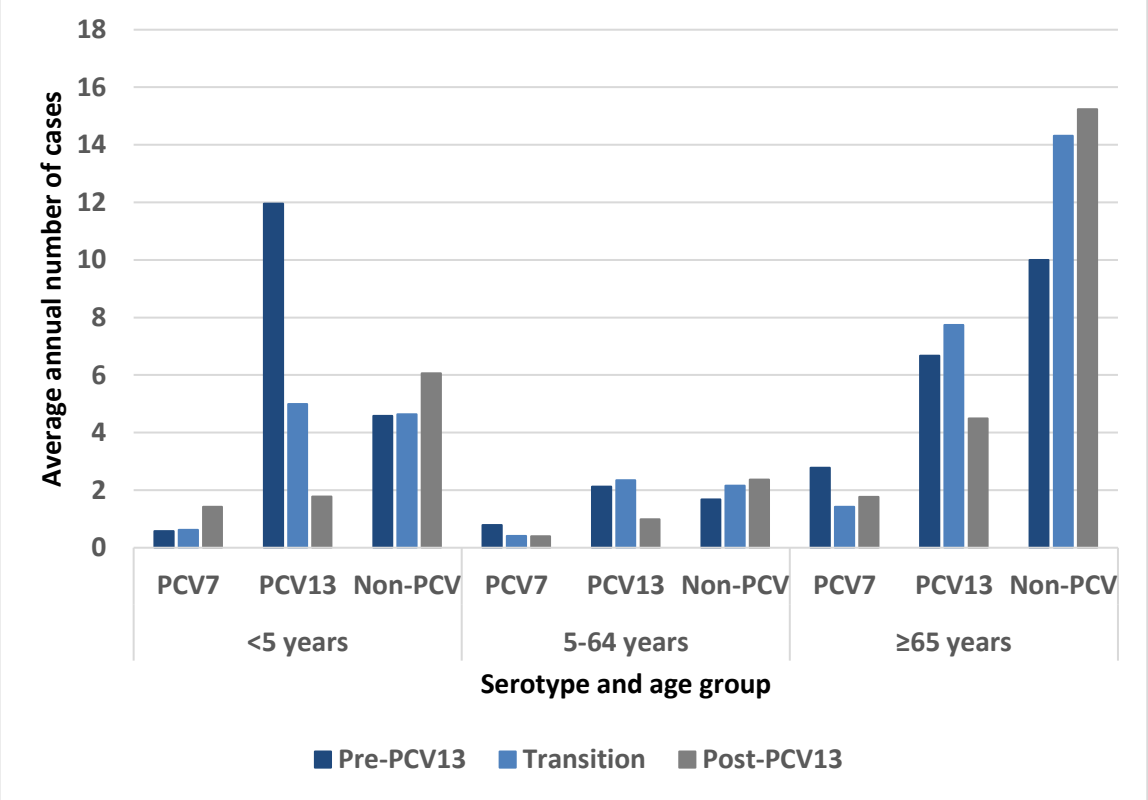

Figure 3. Average annual incidence of IPD by age group and time period, Victoria, 2008 to 2018. Data source: isolates submitted to the Victorian Hospital Pathogen Surveillance Scheme.

After the introduction of the PCV13, the largest decrease in the incidence of IPD occurred for those aged the younger than 5 years (IRR 0.70, 95% CI: 0.55–0.88), with a smaller decrease in those 5 to 64 years (IRR 0.87, 95% CI: 0.80–0.95) (Table 3). Conversely, the incidence of IPD in those aged 65 year and older increased (IRR 1.27, 95% CI: 1.16–1.40).

The overall incidence of PCV13 serotypes decreased to the greatest extent in those younger than 5 years (IRR 0.29, 95% CI: 0.20–0.44), with a smaller decrease in those aged 5 to 64 years (IRR 0.59, 95% CI: 0.50–0.69) and those 65 years and older (IRR 0.62, 95% CI: 0.51–0.76). Notably, the incidence of serotype 19A decreased in all age groups (<5 years: IRR 0.15, 95% CI: 0.08–0.30, 5 to 64 years: IRR 0.26, 95% CI: 0.17–0.39, ≥65 years: IRR 0.28, 95% CI: 0.19–0.41). Against this, the incidence of serotype 3 increased in all age groups (<5 years: IRR 1.72, 95% CI: 0.78–3.79, 5 to 64 years: IRR 1.47, 95% CI: 1.07–2.04, ≥65 years: IRR 1.58, 95% CI: 1.11–2.24).

The overall incidence of PCV7 serotypes increased in those younger than 5 years (IRR 4.50, 95% CI: 1.71–11.86) driven by a significant increase in the incidence of serotype 19F (IRR 3.53, 95%

CI: 1.43–8.75). In contrast, the overall incidence of PCV7 serotypes decreased in those aged 5 to 64 years (IRR 0.59, 95% CI: 0.44–0.79) and 65 years and older (IRR 0.51, 95% CI: 0.36–0.73), despite increases in the incidence of serotype 19F (5 to 64 years: IRR 1.67, 95% CI: 1.02—2.76, ≥65 years: IRR 3.43, 95% CI: 1.53–7.68).

Table 3. Comparison of IPD serotype groups and selected serotypes by time period and age group. Incidence rate ratio (IRR), as determined by Poisson regression, indicates the change in incidence compared to the pre-PCV13 time period as the baseline. Data source: isolates submitted to the Victorian Hospital Pathogen Surveillance Scheme.[6]

Factor	Transition period IRR (95% CI)	Post-PCV13 IRR (95% CI)
<5 years		
IPD overall	**0.65 (0.51–0.82)**	**0.70 (0.55–0.88)**
PCV7	1.96 (0.64–6.01)	**4.50 (1.71–11.86)**
Serotype 19F	1.63 (0.56–4.72)	**3.53 (1.43–8.75)**
PCV13	**0.64 (0.49–0.82)**	**0.29 (0.20–0.44)**
Serotype 3	0.56 (0.18–1.74)	**1.72 (0.78–3.79)**
Serotype 19A	**0.54 (0.39–0.77)**	**0.15 (0.08–0.30)**
Non-PCV	**1.67 (1.24–2.25)**	**2.11 (1.61–2.77)**
Serotype 15A	1.40 (0.09–22.19)	6.62 (0.78–56.07)
Serotype 9N	1.40 (0.20–9.80)	3.97 (0.82–19.37)
5 to 64 years		
IPD overall	0.98 (0.91–1.06)	**0.87 (0.80–0.95)**
PCV7	**0.48 (0.36–0.65)**	**0.59 (0.44–0.79)**
Serotype 19F	0.96 (0.56–1.67)	**1.67 (1.02–2.76)**
PCV13	1.00 (0.88–1.12)	**0.59 (0.50–0.69)**
Serotype 19A	**0.67 (0.52–87)**	**0.26 (0.17–0.39)**
Serotype 3	0.98 (0.69–1.38)	**1.47 (1.07–2.04)**
Non-PCV	**1.26 (1.10–1.44)**	**1.72 (1.52–1.94)**
Serotype 9N	1.71 (0.88–3.33)	**4.64 (2.57–8.39)**
Serotype 15A	**4.94 (1.45–16.87)**	3.53 (0.98–12.76)
≥65 years		
IPD overall	**1.14 (1.06–1.26)**	**1.27 (1.16–1.40)**
PCV7	**0.44 (0.30–0.64)**	**0.51 (0.36–0.73)**
Serotype 19F	**2.39 (1.02–5.56)**	**3.43 (1.53–7.68)**
PCV13	0.91 (0.77–1.09)	**0.62 (0.51–0.76)**
Serotype 19A	**0.73 (0.55–0.97)**	**0.28 (0.19–0.41)**
Serotype 3	1.58 (0.81–1.72)	**1.58 (1.11–2.24)**
Non-PCV	**1.23 (1.10–1.37)**	**1.41 (1.27–1.56)**
Serotype 15A	**3.28 (1.53–7.03)**	**2.36 (1.08–5.17)**
Serotype 9N	**3.78 (1.29–11.03)**	**7.46 (2.69–20.67)**

[6] Statistical significance is defined as $p<0.05$, values meeting statistical significance are bolded. Values indicating an increased incidence are shown in red; those indicating a decrease are shown in green.

Antimicrobial susceptibility

Over the study period, 98.7% of (3,815) isolates had available clinical breakpoint data for penicillin and 91.9% (3,551) had data available for third generation cephalosporins (3GCs). Only 50% of isolates (n=1920) had susceptibility results for vancomycin, 42% (n=1634) had susceptibility results for erythromycin, and 30% (n=1148) had susceptibility results for clindamycin.

Among 66 isolates resistant to penicillin, nine were also resistant to 3GC (13.6%). Of 324 isolates non-susceptible to penicillin, 37 were also non-susceptible to 3GC (11.4%). Between time periods, there was no change in the proportion of isolates resistant or non-susceptible to penicillin, however the proportion of isolates susceptible to penicillin increased (88.8% pre-PCV13 to 91.6% post-PCV13, X^2=5.55, p=0.02) (Table 4). There was no change in the proportion of isolates susceptible, non-susceptible or resistant to 3GC.

Table 4. Proportions of isolates by antimicrobial susceptibility and time period, 01 January 2008 to 30 June 2018. Chi-squared or Fisher's exact test indicates whether there was a change in the proportion of isolates between the time periods. Data source: clinical breakpoint data submitted by diagnostic laboratories to the Victorian Hospital Pathogen Surveillance Scheme.[7][8]

Penicillin						
	Pre-PCV13 n (%)	**Transition n (%)**	**Chi-squared**	**Post-PCV13 N (%)**	**Chi squared**	**Total n (%)**
Susceptible	1,072 (88.8%)	1,183 (88.9%)	X^2=0.003, *p*=0.96	1,170 (91.6%)	X^2=5.55, ***p*=0.02**	3,425 (89.8%)
Non-susceptible	115 (9.5%)	119 (8.9%)	X^2=0.26, *p*=0.96	90 (7.0%)	X^2=3.17, *p*=0.08	324 (8.5%)
Resistant	20 (1.7%)	29 (2.2%)	X^2=0.91, *p*=0.61	17 (1.3%)	X^2=2.70, *p*=0.10	66 (1.7%)
Total	1,207	1331		1,277		3,815
3GC						
Susceptible	1,131 (98.1%)	1,211 (97.6%)	X^2=0.73, *p*=0.39	1,133 (97.9%)	X^2=0.32, *p*=0.57	3,475 (97.9%)
Non-susceptible	19 (1.6%)	24 (1.9%)	X^2=0.28, *p*=0.60	19 (1.6%)	X^2=0.29, *p*=0.59	62 (1.7%)
Resistant	3 (0.3%)	6 (0.5%)	Fisher's exact, *p*=0.51	5 (0.4%)	X^2=0.03, *p*=0.85	14 (0.4%)
Total	1,153	1,241		1,157		3,551

[7] Non-susceptible indicates intermediate antimicrobial susceptibility

[8] Statistical significance is defined as $p<0.05$, values meeting statistical significance are bolded

Among isolates with penicillin resistance and non-susceptibility, serotypes 15A, 19A and 19F were most prevalent (Table 5). Among isolates with 3GC resistance, serotype 19A and 19F predominated, whereas among non-susceptible isolates, serotype 19A, 14 and 15A were most prevalent.

Table 5. Serotypes predominant among isolates with resistance and non-susceptibility to penicillin and 3GC, 01 January 2008 to 30 June 2018. Data source: Victorian Hospital Pathogen Surveillance Scheme.

	Penicillin		3GC	
Serotype	**Resistant**	**Non-susceptible**	**Resistant**	**Non-susceptible**
Serotype 4	1 (1.5%)	2 (0.6%)	0	0
Serotype 6A	1 (1.5%)	3 (0.9%)	0	0
Serotype 7F	0	0	1 (7%)	2 (3.2%)
Serotype 11A	2 (3.0%)	13 (4.0%)	0	2 (3.2%)
Serotype 12F	1 (1.5%)	1 (0.3%)	0	0
Serotype 14	4 (6.1%)	16 (4.9%)	0	8 (12.9%)
Serotype 15A	11 (16.7%)	37 (11.4%)	0	7 (11.3%)
Serotype 15B	1 (1.5%)	7 (2.2%)	0	0
Serotype 15C	2 (3.0%)	2 (0.6%)	0	0
Serotype 19A	19 (28.8%)	120 (37.0%)	4 (29%)	18 (29.0%)
Serotype 19F	8 (12.1%)	9 (2.8%)	4 (29%)	4 (6.5%)
Serotype 20	1 (1.5%)	1 (0.3%)	0	0
Serotype 23A	2 (3.0%)	21 (6.5%)	0	0
Serotype 23B	1 (1.5%)	18 (5.6%)	1 (7%)	5 (8.1%)
Serotype 23F	1 (1.5%)	5 (1.5%)	0	2 (3.2%)
Serotype 33F	0	7 (2.2%)	0	0
Serotype 35B	4 (6.1%)	18 (5.6%)	1 (7%)	6 (9.7%)

Penicillin MIC data were available for 2441 isolates (63.2%); 3GC MIC data were available for 2221 isolates (57.5%). Regression analysis showed a 25% increase in the incidence (IRR 1.25, 95% CI: 1.04–1.51) of raised penicillin MIC (>0.06 mg/L) in the transition period, however there was no change in the post-PCV13 period (IRR 1.02, 95% CI: 0.84–1.24). For 3GC with raised MIC (≥0.5 mg/L), there was no statistically significant change in either the transition or post-PCV13 period (IRR 1.15, 95% CI: 0.81–1.63, IRR 1.11, 95% CI: 0.78–1.59, respectively).

Discussion

Since the introduction of the PCV13, there has been a significant decrease in the overall incidence of vaccine serotypes in Victoria. Against this, the incidence of serotypes 3 and 19F have increased in all age groups, despite their inclusion in the PCV13. Based on clinical

breakpoint data reported by Victorian diagnostic laboratories, the proportion of isolates susceptible to penicillin increased, and the levels of AMR to 3GC were comparable.

The overall rate of IPD remained highest in individuals younger than 5 years and 65 years of age and older, as in other countries (19-21). In the post-PCV13 period, a 46% reduction in the incidence of PCV13 serotypes was largely driven by decreases in serotype 1, 6A and 19A. While the PCV13 is funded for children younger than 5 years, the incidence of PCV13 serotypes decreased in all age groups, as in other studies (5, 22-26). While we hypothesised that the incidence of all vaccine serotypes would decrease after the introduction of the PCV13, there was a significant increase in the incidence of serotype 3 and serotype 19F in the post-PCV13 period. This is consistent with other studies examining serotype changes after the introduction of the PCV13 (24, 27). Other studies found no decrease in serotype 3 after the introduction of PCV13 (8, 28, 29). Serotypes 3 and 19F have both been associated with increased risk of death (30). Serotype 19F has also been associated with penicillin and cefotaxime resistance and was one of the most prevalent serotypes displaying AMR in this analysis (11).

It is possible that the increase in serotype 19F occurred due to reduced vaccine efficacy against this serotype (19, 31, 32). A review of vaccine failures in children found that 19F had one of the highest incidents of vaccine failure (33). This analysis was not able to examine this possibility, as no vaccination records are collected by the VHPSS. To further investigate the effectiveness of the PCV13 against serotype 19F, additional studies including factors such as number of doses and age at which they were received would be required (34).

While PCV13 appears to have efficacy against serotype 3 at the individual level in both children and adults, it does not appear to be effective at the population level (35). In those aged 65 years and older, serotype 3 was the second most-prevalent, which may be due to minimal herd immunity for this serotype (7, 31, 36). As colonisation with *S. pneumoniae* is a precursor to IPD, and children younger than 5 years are a major source of transmission for adults, herd immunity relies on the prevention of carriage of PCV13 serotypes in children (37). The persistence of serotype 3 may therefore be explained by PCV13 being successful at directly protecting against disease, but not as effective at preventing carriage (7, 35). If colonisation with serotype 3 persists among children, it follows that this serotype can be transmitted and potentially be an increasing cause of IPD among adults and the unvaccinated (35). Rather than relying on herd immunity for serotype 3, direct vaccination of populations at risk of disease, using the PCV13, is likely to be a more successful approach to reducing disease due to this serotype (7, 24, 29, 35, 37).

Consistent with other studies, non-PCV serotypes increased after the introduction of the PCV13, comprising more than half of all cases over the study period (11, 23, 38, 39). The most

pronounced increases in incidence were seen in serotype 9N, 15A and 15C. Serotype 15A has emerged in several countries following the implementation of PCV13 (14, 40-42). Increases in the incidence of serotype 15A have been associated with meropenem non-susceptibility, penicillin non-susceptibility, macrolide resistance, and multi-drug resistance (14, 41-44). The prevalence of AMR among isolates of serotype 15A is concerning, as this is not included in any of the conjugate vaccines or the 23vPPV used in Australia (44).

Post-PCV13 proportions of penicillin non-susceptibility and resistance are low compared to other studies however somewhat higher than penicillin resistance among invasive isolates of *S.pneumoniae* in the UK (0.7%) (5, 8, 11, 21, 45, 46). Low levels of resistance over the study period indicate continued use of penicillin and 3GC is appropriate for empirical treatment of IPD in Victoria. While some studies assessing the impact of the PCV13 have found reductions in the proportion of isolates resistant to penicillin or cefotaxime, others have found no change in AMR with the emergence of non-vaccine serotypes (5, 23, 24, 47). Others identified increases in penicillin resistance among non-vaccine serotypes (35). While proportions of isolates with penicillin resistance were comparable between pre- and post-PCV13 periods, the proportion susceptible to penicillin increased. This small change in the prevalence of penicillin susceptibility is unlikely to be clinically significant. This change was driven by a significant decrease in the incidence of serotype 19A, among all age groups, which is commonly associated with high levels of penicillin non-susceptibility (24).

This analysis used data collected by the VHPSS, which relies on voluntary contribution by diagnostic laboratories. While the majority of isolates notified to the DHHS appear to have been included in this study, there are several limitations with available data. This analysis was only able to examine antimicrobial susceptibility data for penicillin and 3GC, which are used for empirical treatment of IPD in Australia. To obtain a more comprehensive understanding of the changes in the antimicrobial susceptibility of *S. pneumoniae*, additional antimicrobials should be included in future analyses. Diagnostic laboratories contributing to the VHPSS test *S. pneumoniae* for a range of antimicrobials however there is a lack of consistency of testing and reporting between laboratories. As a large proportion of MIC results were missing from the dataset, useful conclusions on trends in MIC values were not able to be obtained from this analysis. Furthermore, AST techniques and guidelines used to interpret them are not consistently reported, indicating that there is a need for standardisation of AST reporting to the VHPSS.

From July 2018, the vaccine schedule for children under the National Immunisation Programme changed from 3 + 0 to 2 + 1. This is expected to lead to further changes in the epidemiology of

IPD, therefore similar analyses, with the addition of MIC and vaccination data, should be conducted in future.

Conclusions

This study identified an overall decrease in PCV13 serotypes after the introduction of PCV13 in Victoria. This was partly offset by increases in non-PCV13 serotypes. There was no increase in AMR to penicillin or 3GC antimicrobials. These findings indicate the importance of ongoing surveillance to identify changes in the epidemiology of serotypes and AMR within IPD in Victoria.

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Appendix 1. Poster presentation at the AEA Conference, Brisbane, October 2019

Invasive pneumococcal disease: Changes in epidemiology following introduction of the 13-valent pneumococcal conjugate vaccine, Victoria, 2008–2018

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Introduction

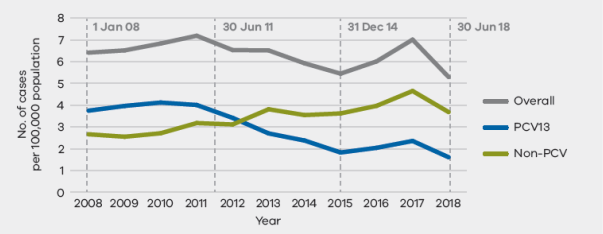
- Invasive pneumococcal disease (IPD) has been notifiable in Victoria since 2001. Serotype data are also available through the Victorian Hospital Pathogen Surveillance Scheme (VHPSS), a laboratory-based surveillance system that monitors bacterial pathogens from blood and cerebrospinal fluid samples in Victoria. The VHPSS is coordinated within the Microbiological Diagnostic Unit – Public Health Laboratory (MDU-PHL) at the Peter Doherty Institute for Infection and Immunity.
- In 2005 a pneumococcal conjugate vaccine that included seven serotypes (PCV7) was funded under the National Immunisation Program for infants vaccinated at two, four and six months of age.
- In July 2011 the 13-valent pneumococcal conjugate vaccine (PCV13), including an additional six serotypes, replaced the PCV7.
- **Study aim:** To assess changes in pneumococcal serotypes for IPD in Victoria in the period following PCV13 introduction.

Methods

- Data were extracted from the VHPSS for all cases of IPD from 1 January 2008 to 30 June 2018.
- Isolates were divided into three equal time periods: **pre-PCV13:** 1 January 2008 to 30 June 2011; **transition:** 1 July 2011 to 31 December 2014; and **post-PCV13:** 1 January 2015 to 30 June 2018.
- Statistical analysis was conducted using Stata 15.1. Poisson regression was used to assess changes in the incidence of serotypes between pre and post PCV13.
- Incidence rates were calculated using the Australian Bureau of Statistics Estimated Resident Populations in relevant years.

Incidence of IPD: Victoria, 2008–2018

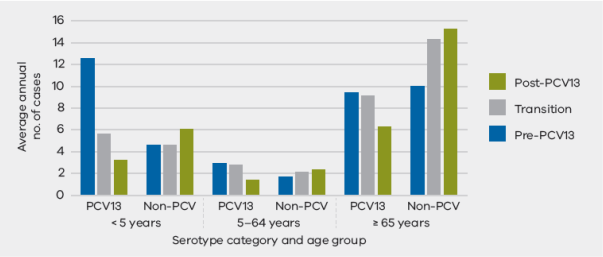
Figure 1: Incidence rate of IPD per 100,000 population per year, Victoria, 1 January 2008 to 30 June 20181



1 While the data suggest there has been a decrease in the incidence of IPD overall in 2018, this is due to seasonality. The highest number of notifications of IPD usually occur in quarter 3 (July to September).

The largest reduction in PCV13 cases occurred in the under five-year age group; however, decreases were seen in all age groups (Figure 2).

Figure 2: Average annual number of cases by serotype category, age group and time period, 1 January 2008 to 30 June 2018, Victoria



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Changes with the introduction of PCV13

While there was a 46 per cent reduction in PCV13 serotypes after the introduction of PCV13, there was a 63 per cent increase in non-PCV serotypes (Table 1). Despite being included in the PCV13, serotype 3 and 19F increased.

Table 1: Comparison of IPD serotypes by time period
Incidence rate ratio (IRR) indicates the changes in incidence post-PCV13 compared with the pre-PCV13 period[1,2]

PCV13 serotypes	IRR	Non-PCV serotypes	IRR
Serotype 1*	**0.07 ↓**	Serotype 6C	0.77 ↓
Serotype 3*	**1.56 ↑**	Serotype 9N	**5.07 ↑**
Serotype 4	**0.15 ↓**	Serotype 11A	1.45 ↑
Serotype 5*	–	Serotype 15A	**3.16 ↑**
Serotype 6A*	**0.04 ↓**	Serotype 16F	**2.09 ↑**
Serotype 6B	**0.26 ↓**	Serotype 22F	0.89 ↓
Serotype 7F*	**0.54 ↓**	Serotype 23A	**2.19 ↑**
Serotype 9V	**0.20 ↓**	Serotype 23B	**2.68 ↑**
Serotype 14	**0.50 ↓**	Serotype 33F	1.36 ↑
Serotype 18C	**0.07 ↓**	Serotype 35B	1.26 ↑
Serotype 19A*	**0.23 ↓**	Others	**1.86 ↑**
Serotype 19F	**2.18 ↑**		
Serotype 23F	**0.25 ↓**		
Total	**0.54 ↓**	**Total**	**1.63 ↑**

1 Statistical significance is defined as $p < 0.05$. Values meeting statistical significance are shown in bold blue font.
2 "Other" serotypes include those that make up less than 2 per cent of the total (33 serotypes not listed).
* Indicates the six additional serotypes added to the PCV7 to make the PCV13.

Conclusions

There has been a significant decrease in the overall incidence of PCV13 serotypes in Victoria after the introduction of the PCV13. Further investigation is necessary to determine why serotypes 19F and 3 have continued to increase.

The overall decrease has been partly offset by increases in the incidence of non-vaccine serotypes. Ongoing surveillance is required to identify changing serotypes and inform future vaccine development.

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Chapter III: Surveillance of Antimicrobial Resistance in Victoria

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1. Preface

There are multiple systems that conduct surveillance of antimicrobial resistance (AMR), both within Victoria and Australia-wide. While these systems enable AMR trends to be examined, the data that the systems produce currently lack integration. Furthermore, available data are not always accessible to the Victorian Government Department of Health and Human Services (DHHS) which has the legislative power and authority to implement public health response to increasing levels of AMR or an increase in cases with a particular AMR pattern.

Marion Easton, the Principal Epidemiologist for AMR and Whole Genome Sequencing (WGS), initiated this project as a result of plans to develop an AMR Surveillance and Response Unit within the DHHS. This unit is referred to as the Victorian AMR unit throughout this chapter. Since this evaluation commenced, the Victorian AMR unit has been established, however is still in the early stages of operation. The primary aim of this project was to determine how the surveillance of AMR within Victoria could be improved.

1.1 My role

I developed the evaluation framework for this project, conducted document reviews for current systems monitoring AMR in Victoria and Australia, designed semi-structure stakeholder interview guides and an online survey in Qualtrics, conducted the interviews, collected and analysed data, and made recommendations.

1.2 Acknowledgements

I acknowledge the following people and organisations for their assistance and participation in this project:

- Marion Easton and Professor Benjamin Howden, for sharing their experiences of working on AMR in Victoria, and for assisting in refining the project aims and questionnaire design
- Siobhan St George, Courtney Lane, Mary Valcanis, and Kerrie Stevens for sharing their experiences of AMR surveillance at the Microbiological Diagnostic Unit Public Health Laboratory (MDU)
- Dr Emma Field for her valuable feedback on structuring and refining this evaluation
- Dr Norelle Sherry for sharing her knowledge on AMR surveillance in Victoria

- All Victorian diagnostic laboratories who discussed their experiences of AMR surveillance in Victoria
- The coordinators of The Australian Group on Antimicrobial Resistance for sharing their experiences of AMR surveillance

1.3 Lessons learnt

Through conducting this project, I have learnt about real-world public health surveillance in addition to how to conduct a surveillance system evaluation. The evaluation process has enabled me to gain an understanding of the complexities of antimicrobial susceptibility testing (AST), laboratory processes, and AMR surveillance techniques and limitations. In the second year of my MAE, I was responsible for entering eligible isolates into the National Critical Antimicrobial Resistances Alert system on behalf of MDU. This gave me direct experience of AMR surveillance, and enabled me to further understand AMR and WGS testing processes.

The stakeholder engagement process was conducted via a combination of in-person interviews and a web-based survey. Using a combination of methods taught me the advantages and disadvantages of each. I learnt that semi-structured interviews are time consuming and can be difficult to arrange yet enable issues to be investigated in greater detail. In contrast, online surveys are challenging to design and carry the risk of misinterpretation by the respondent, however, are efficient and quick, and enabled the maximum number of stakeholders to contribute. In future, I would commence stakeholder engagement earlier and conduct in-person interviews followed by online surveys to obtain additional information.

Lastly, the complexity of AMR surveillance has highlighted the importance of refining project objectives on commencement of the evaluation process. AMR surveillance is a very broad topic, encompassing all bacterial and fungal organisms when considered at the highest level. Each organism has different considerations within the laboratory-based surveillance system, including which antimicrobials are tested and monitored, the use of WGS results, and the collection of specific patient variables. Once AMR surveillance is further established within the DHHS, future evaluations should focus on assessing the surveillance of AMR within one or two organisms. Conducting evaluations with a narrower focus will enable more specific recommendations to be made.

1.4 Public health implications

This evaluation reviewed the current surveillance activities for AMR in Victoria and highlighted the differences and interactions between systems. Current AMR surveillance activities in Victoria are successful in monitoring long term trends, however surveillance lacks the timeliness to implement a useful public health response to outbreaks or emerging resistance for most organisms. In addition, many of the existing surveillance systems do not currently complete the surveillance cycle through providing timely and useful reports to relevant stakeholders.

Recommendations to improve the data quality, timeliness, acceptability and usefulness of AMR surveillance in Victoria were formulated. Importantly, the implementation of reporting to end-users of AMR data is essential to enable effective public health action for AMR. This evaluation highlighted that AMR surveillance is currently conducted in a resource-limited setting. Future surveillance activities must therefore be prioritised through only conducting continuous surveillance for specific organism-antimicrobial combinations, with AMR in other organisms potentially monitored using intermittent cross-sectional surveys.

Another key issue with current AMR surveillance processes is the potential for duplication of effort between systems. This could be improved by establishing a clear governance structure and documentation of processes for AMR surveillance. Publication of an AMR surveillance strategy for Victoria to outline governance would assist in clarifying which of the existing systems is responsible for selected organism-antimicrobial combinations. In this way, AMR surveillance could be conducted more effectively and potentially for a wider range of organisms.

2. Abstract

Introduction: AMR occurs when bacteria or fungi survive in the presence of antimicrobial agents. Organisms with AMR cause infections that are more difficult to treat, and are associated with higher morbidity and mortality. Moreover, AMR is associated with longer hospital stays and increased healthcare costs. Currently, there are several surveillance systems monitoring AMR in Victoria, however these systems lack integration. A centrally coordinated Victorian AMR unit, based at the DHHS, is expected to improve the integration and public health impact of AMR surveillance in Victoria. To inform the implementation of this surveillance unit, an evaluation of existing surveillance activities in Victoria was conducted.

Methods: This evaluation followed the Centers for Disease Control and Prevention's *Updated Guidelines for Evaluating Public Health Surveillance Systems*. Attributes evaluated included simplicity, data quality, acceptability, representativeness, timeliness and usefulness. Attributes were evaluated using a combination of document review and interviews with surveillance coordinators and contributing diagnostic laboratories.

Results: Victorian diagnostic laboratories currently contribute to 10 AMR surveillance systems. Four systems conduct surveillance in Victoria: the National Enteric Pathogen Surveillance Scheme, the Victorian AMR unit, the Victorian Health-Care Associated Infection Surveillance System, and the Victorian Hospital Pathogen Surveillance Scheme. Victoria also contributes to six systems at the national level: the Australian Group on Antimicrobial Resistance, the Australian Passive AMR Surveillance System, the Antimicrobial Use and Resistance Australia Surveillance System, the National Critical Antimicrobial Alert Surveillance System, the National Neisseria Network, and the National Notifiable Diseases Surveillance System.

Existing AMR surveillance in Victoria is complex, in part due to the number of systems involved. The quality of data collected by AMR surveillance systems is high, however there are issues with the standardisation of antimicrobial susceptibility data. Several factors affect the representativeness of AMR surveillance in Victoria. In addition, Victoria is underrepresented in national AMR surveillance. Diagnostic laboratories do not find current surveillance activities highly acceptable, as current methods of data submission are time consuming and resource intensive. Furthermore, several systems are not currently producing reports on surveillance activities. While current AMR surveillance is useful for monitoring long term trends in Victoria, it does not enable a public health response to be conducted in a timely manner.

Recommendations and conclusion: While current AMR surveillance in Victoria successfully monitors long-term trends, it suffers from a lack of co-ordinated governance and oversight and

centralisation of data collection. Improvements in the dissemination of surveillance data, timeliness of data sharing, and reporting would greatly improve the public health impact of AMR surveillance in Victoria. Key recommendations include the development of a Victorian AMR surveillance strategy, with clear documentation of surveillance processes and prioritisations, and the implementation of regular, timely and useful reporting to relevant stakeholders.

3. Introduction

3.1 What is AMR?

AMR is defined as reduced effectiveness of antimicrobials against microbial organisms (1). The terms AMR and antibiotic resistance are often used interchangeably. As this chapter focuses exclusively on bacteria, the term AMR will be used to refer to antibiotic resistance, the resistance of bacteria to antibiotics.

Antibiotics target specific components of bacterial cells and are grouped into classes based on the mechanism of action, such as inhibition of cell wall production (e.g. penicillin), protein production (e.g. tetracyclines) and DNA production (e.g. fluoroquinolones) (2). Many bacteria have intrinsic or natural AMR. Intrinsic AMR may occur as a result of the bacteria not possessing a specific cellular target, the antimicrobial not being able to access the cellular target, or due to the bacteria's production of enzymes which inactivate the antimicrobial, such as beta-lactamase which inactivates penicillin (1, 3). Such enzymes are often encoded by plasmids, which are circular pieces of DNA located in the cell cytoplasm, and are able to be transmitted between bacteria (4, 5).

In addition to intrinsic AMR, bacteria can acquire resistance mechanisms through genetic mutations or the transfer of resistance genes from other organisms (6). All humans carry normal or commensal gut flora, which can acquire genes conferring AMR. Bacteria with acquired resistance have a selective advantage over bacteria susceptible to a particular antibiotic (5, 7). Excessive and indiscriminate use of antimicrobials acts as a selection pressure for bacteria, enabling bacteria with resistance to survive in preference to susceptible bacteria, contributing to increasing prevalence of AMR (5, 7).

3.2 The spread of AMR

In addition to transferring resistance genes to other organisms, resistant bacteria can spread between infected or colonised people, both within the community and healthcare facility setting (8). Within healthcare facilities, infections can spread directly between patients, via contact with health-care workers or other people, and indirectly through contaminated environmental surfaces including medical equipment and hospital furniture (8). In contrast, in the community, transmission of resistant bacteria often occurs through faecal-oral or airborne transmission and via sexual contact (3). Close relationships between humans and animals provide another potential pathway for the transmission of resistance mechanisms or resistant organisms and transmission of resistance may occur through food derived from animals (9, 10). Resistant

bacteria within humans and animals can also be excreted into the environment and enter waterways via the sewage system (11).

International travel, particularly that to and from Asia and India, is associated with an increased risk of becoming colonised by an antibiotic resistant bacteria, especially when individuals have been hospitalised overseas (4). With the increasing frequency and availability of international travel and trade, the likelihood of resistant organisms being transferred across borders has increased (12). In addition, as Australia is located in close proximity to many countries with less strict antimicrobial prescribing practices and regulations, it is increasingly at risk of cross-border transmission of multi-resistant organisms (13).

3.3 Public health importance of AMR

It is estimated that antibiotic-resistant bacterial infections cause at least 700,000 deaths annually, which may increase to 10 million deaths by the year 2050 (7). No new antimicrobial classes have been developed since the 1980s, therefore action must ensure that the available antimicrobials remain effective (12).

The World Health Organization states that we may move into a “post-antibiotic era” if we do not take action to prevent increasing levels of AMR, as modern medicine heavily relies on the use of antimicrobials to treat infectious diseases (7, 12). Infections caused by organisms with AMR are more difficult to treat successfully and are associated with increased morbidity and mortality (12, 14). Furthermore, the availability and success of chemotherapy and complex surgical procedures is dependent on the use of antimicrobials to treat infections which may arise from these treatments (5, 7). Increasing AMR is also associated with higher healthcare costs due to the requirement for newer antimicrobials, increased treatment durations and longer stays in hospital, as well as increased workloads within the healthcare system (14).

3.4 Surveillance of AMR

The Centers for Disease Control and Prevention defines surveillance as “the ongoing, systematic collection, analysis, interpretation, and dissemination of data regarding a health-related event for use in public health action to reduce morbidity and mortality and to improve health” (15).

The surveillance of AMR relies on the results of AST which is conducted by microbiology laboratories. Common testing techniques for bacteria include automated broth microdilution, such as Vitek, disc diffusion and E tests (Appendix 1) (1). These tests produce a minimum

inhibitory concentration (MIC), which is the concentration of the level of antimicrobial required to inhibit the growth of the bacteria being tested (1).

The results of AST are interpreted using guidelines which are published by organisations such as the US Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), which are the two major standards used globally (1). These guidelines define the clinical breakpoints for each organism and antimicrobial combination, which are the levels of MICs defined as susceptible, intermediate and resistant (1).

Surveillance is required to determine the prevalence of AMR, identify the emergence of new resistance patterns, assess changes in AMR, identify outbreaks, and to inform the most appropriate control actions (13). The results of AMR surveillance can also inform antimicrobial stewardship within jurisdictions (13). Antimicrobial stewardship programs, both within hospitals and the community, seek to reduce indiscriminate prescribing of broad-spectrum antimicrobials by practitioners, and to ensure appropriate antimicrobials are used to treat infections (5).

Several countries have well developed surveillance systems for AMR. For example, the European Centre for Disease Control has coordinated the European Antimicrobial Resistance Surveillance Network since 2005, which conducts AMR surveillance spanning multiple European countries. The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme has conducted continuous monitoring of AMR and antimicrobial use in animals, food and humans since 1995, with the program resulting in decreased levels of AMR (16).

In Australia, the Australian Commission on Safety and Quality in Health Care has implemented the national Antimicrobial Use and Resistance Australia and National Critical Antimicrobial Resistance Alert (CARAlert) surveillance systems. These systems are successful in monitoring national AMR trends, in combination with antimicrobial use, however do not enable a timely response to emerging AMR or outbreaks.

3.5 Aims of the evaluation

The aim of this evaluation was to identify ways in which existing AMR surveillance activities, coordination and governance of AMR surveillance in Victoria could be improved, in order to inform the implementation of an AMR surveillance and response unit within the DHHS.

3.6 Scope of the evaluation

The systems evaluated in this chapter were assessed in terms of AMR surveillance only, although many of these systems monitor other components such as serotype prevalence and antimicrobial use. While surveillance of antimicrobial use, in combination with surveillance of AMR, is an essential component of the public health response to AMR, an evaluation of this aspect of surveillance was beyond the scope of this project (3). While a one health approach to AMR surveillance, incorporating human, animal, food and environmental isolates, is essential, evaluation of this aspect of surveillance was also considered beyond the scope of this project (9).

AMR surveillance is important for many organisms, however this evaluation focussed only on those in Table 1. Organisms considered were those that are notifiable in Victoria, with existing or emerging AMR of clinical and public health importance, and those included in the CARAlert system (Table 1). A distinction is made between notifiable and non-notifiable health-care acquired organisms as the legislative power for follow up of cases by the DHHS differs. The DHHS routinely follows up AMR within notifiable diseases within healthcare facilities, however cannot follow up non-notifiable diseases unless they are deemed reportable under the *Victorian Public Health and Wellbeing Act 2008.* For example, while carbapenemase-producing Enterobacterales (CPE) is not notifiable in Victoria, reporting of all cases to the DHHS is performed according to the *Victorian guideline on CPE for Health Services.* At the time of writing, the regulations of the *Victorian Public Health and Wellbeing Act 2008* were under review. New regulations mean that additional organisms will become notifiable as of 2020, including: CPE, Carbapenemase-producing *Pseudomonas* sp. and *Acinetobacter* sp., *van*A vancomycin-resistant enterococci, and *Candida auris*.

Table 1. Organisms considered in the evaluation

Organism	Notifiable pathogen[1]	National Critical Antimicrobial Resistance Alert Surveillance System (CARAlert)
Carbapenemase-producing Enterobacterales	Will be notifiable as of 2020	Yes
Enterococcus spp.	*van*A vancomycin-resistant enterococci will be notifiable as of 2020	Yes
Haemophilus influenzae type b	Yes	Not included in the CARAlert system
Invasive *Streptococcus pneumoniae*	Yes	Not included in the CARAlert system
Neisseria gonorrhoeae	Yes	Yes
Neisseria meningitides	Yes	Not included in the CARAlert system
Salmonella spp.	Yes	Yes
Shigella spp.	Yes	Yes
Streptococcus pyogenes	May become notifiable in future	Yes

[1] Notifiable diseases are those that are mandated to be reported to the DHHS under the regulations of the *Victorian Public Health and Wellbeing Act 2008*

3.7 Evaluation objectives

The objectives of this evaluation were to:

- Describe current surveillance activities for AMR in Victoria, and national activities to which Victoria contributes;
- Evaluate selected attributes of AMR surveillance in Victoria; and
- Identify possible areas for improvement of surveillance of AMR in Victoria, given available resources

4. Methods

This evaluation was conducted using the Centers for Disease Control and Prevention's *Updated Guidelines for Evaluating Public Health Surveillance Systems* (15) as a guide. A framework for evaluation of AMR surveillance in Victoria was developed using the relevant attributes. Surveillance systems for the evaluation were identified in consultation with the DHHS Principal Epidemiologist for AMR and WGS, and through review of AMR surveillance annual reports. Stakeholders for consultation were identified through interviews with staff at MDU and the DHHS (Table 2).

Table 2. Stakeholders and contribution to the evaluation of AMR surveillance in Victoria

Organisation	Stakeholder or Informant	Contribution to the evaluation
The Australian Group for Antimicrobial Resistance	Chairperson and scientific officer	Discussion of experiences setting up AMR surveillance and coordinating data collection
MDU	Epidemiologists (surveillance coordinators of the Victorian Hospital Pathogen Surveillance Scheme, National Enteric Pathogen Surveillance Scheme, Victorian CPE Surveillance and Response Unit)	Discussion of the function and experiences of existing systems coordinated within MDU
	Laboratory managers	Discussion of the details of AST, data sharing between DHHS and MDU, and contributions to existing systems
Victorian diagnostic laboratories	Senior scientists Clinical microbiologists	Discussion of experiences of existing surveillance systems for AMR, providing details on data sharing processes and AST
DHHS	Principal Epidemiologist for AMR and WGS	Formulation of objectives for AMR surveillance, discussion of experiences of existing systems
	Partner Notification Officer manager	Discussion of existing follow up procedures

Selected attributes reviewed were simplicity, data quality, acceptability, representativeness, timeliness and usefulness. Stability, sensitivity, flexibility and positive predictive value were not evaluated. Stability and flexibility were not considered to be relevant to the aims of the evaluation and positive predictive value and sensitivity are not possible to evaluate within the context of this project. The attributes were evaluated via document review of surveillance protocols, interviews with MDU epidemiologists and laboratory staff, and where available, coordinators of surveillance systems (Table 3). Assessment of the data structure of each surveillance system was not performed as part of this evaluation.

As key stakeholders, Victorian diagnostic laboratories contributed to this review. As there are more than 20 diagnostic laboratories in Victoria, the decision was made to consult with a single representative from each organisation (n=12). Senior staff at diagnostic laboratories were given the option to discuss their experiences of AMR surveillance in-person or by telephone interview, or by completion of a questionnaire. Due to stakeholder time constraints, six laboratories elected to complete a written questionnaire.

4.1 Ethics approval

A waiver of consent under the Australian National University Human Research Ethics Committee protocol 2017/909 covered this study.

Table 3. Data sources for evaluation and attributes assessed for each AMR surveillance system to which Victorian laboratories contribute

Surveillance system	Interview with surveillance coordinator	Interview with laboratories	Document review	Attributes evaluated					
				Simplicity	Data quality	Acceptability	Representativeness	Timeliness	Usefulness
Australian Group on Antimicrobial Resistance (AGAR)	✓	✓	✓	✓	✓	✓	✓	✓	✓
Australian Passive AMR Surveillance System (APAS)			✓	✓		✓	✓		
Antimicrobial Use and Resistance Australia (AURA)		✓	✓	✓			✓	✓	✓
National Alert System for Critical Antimicrobial Resistances (CARAlert)		✓	✓	✓	✓	✓	✓	✓	✓
National Enteric Pathogen Surveillance Scheme (NEPSS)	✓								✓

National Neisseria Network (NNN)		✓	✓		✓		✓	✓	✓
National Notifiable Disease Surveillance System (NNDSS)		✓	✓	✓	✓	✓	✓	✓	✓
Victorian AMR unit	✓	✓	✓	✓	✓	✓	✓	✓	✓
Victorian Hospital Pathogen Surveillance System (VHPSS)	✓	✓	✓	✓	✓	✓	✓	✓	✓
Victorian Health Care Acquired Pathogen Surveillance System (VICNISS)			✓						✓

5. Results

5.1 Description of surveillance activities

In total, there are 10 surveillance systems to which Victorian laboratories contribute (Table 4). Of these systems, four are coordinated in Victoria: the National Enteric Pathogen Surveillance system (NEPSS), the Victorian AMR unit (including the Victorian Tuberculosis Programme and the Victorian CPE Surveillance and Response Unit (VCRSU)), the Victorian Hospital Pathogen Surveillance Scheme (VHPSS), and the Victorian Health-Care Associated Infection Surveillance System (VICNISS). Six of the 10 surveillance systems are coordinated nationally: the Australian Group on Antimicrobial Resistance (AGAR), the Australian Passive AMR Surveillance System (APAS), Antimicrobial Use and Resistance Australia (AURA), the National Alert System for Critical Antimicrobial Resistances (CARAlert), the National Notifiable Diseases Surveillance System (NNDSS), and the National Neisseria Network (NNN).

Table 4. Organisms included in AMR surveillance systems[2]

	Victorian Systems				National Systems					
Organism	NEPSS	Victorian AMR unit	VHPSS	VICNISS	AGAR	APAS	AURA	CARAlert	NNDSS	NNN
CPE[3]		✓	✓		✓	✓	✓	✓		
Enterococcus spp.			✓	✓	✓	✓	✓	✓		
H. influenzae type b			✓			✓	✓			
Invasive *S.pneumoniae*			✓			✓	✓		✓	
M. tuberculosis		✓					✓	✓	✓	
N. gonorrhoea		✓					✓	✓		✓
N. meningitides			✓				✓			✓
Salmonella sp.	✓	✓	✓			✓	✓	✓		
Shigella sp.	✓	✓				✓	✓	✓		
S. aureus			✓	✓	✓	✓	✓	✓		
S. pyogenes			✓			✓	✓	✓		

[2] The NNDSS, which collates notifications of disease from all jurisdictional health departments, is in the process of developing data specifications for the surveillance of AMR for gonorrhoea, to commence later in 2019

[3] Carbapenemase-producing Enterobacterales

5.1.1 AGAR

AGAR has conducted surveillance of AMR since 1985, initially focusing on *S. aureus* (17). The focus of surveillance has evolved, and AGAR currently conducts national laboratory-based surveillance of AMR within blood-stream infections. Funded by the Australian Commission for Safety and Quality in Health Care, surveillance currently focuses on three surveys (Table 5). In Victoria, only five large laboratories based within healthcare facilities contribute to AGAR. Participating laboratories submit data to AGAR via a web portal and send isolates for typing to laboratories based in Western Australia and South Australia.

Table 5. AGAR surveillance programs (18-20)

Surveillance Program	Organisms	Focus of surveillance
Australian Enterococcal Sepsis Outcome Programme	*Enterococcus* spp.	Ampicillin and glycopeptide resistance molecular epidemiology
Australian Staphylococcal Sepsis Outcome Programme	*S. aureus*	Methicillin resistance Molecular epidemiology of methicillin resistant *S. aureus*
Gram-Negative Sepsis Outcome Programme	Enterobacterales *Pseudomonas aeruginosa* *Acinetobacter* spp.	Multi-drug resistance Emerging resistance to carbapenems and colistin Molecular epidemiology

5.1.2 APAS

APAS was established in 2015 by the Australian Commission for Quality and Safety in Health Care, supported by Queensland Health (21). APAS is a national sentinel surveillance system for AMR. APAS uses a data cube which accepts data extractions from laboratory information systems. Once a laboratory is set up with the system, they can access the data cube to prepare reports and antibiograms, which are profiles of AST results used to contribute to improved antimicrobial stewardship within healthcare facilities. In Victoria, only one laboratory based at Monash Health currently contributes to APAS.

5.1.3 AURA

AURA was established in 2014 by the Australian Commission on Safety and Quality in Health Care (22). AURA was implemented to conduct surveillance of AMR and antimicrobial use at the

national level (23). Surveillance focuses on a defined set of priority organisms with data collated from several different sources (Table 6).

Table 6. AURA data sources (22)

Organism	Data source
Carbapenemase-resistant Enterobacterales	AGAR, APAS, CARAlert
Enterococcus spp.	AGAR, APAS, CARAlert
H. influenzae type b	APAS
M. tuberculosis	CARAlert, NNDSS
N. gonorrhoeae	CARAlert, NNN
N. meningitides	NNN
Salmonella spp.	AGAR, APAS, CARAlert
Shigella spp.	AGAR, APAS, CARAlert
S. aureus	AGAR, APAS, CARAlert
S. pneumoniae	APAS
S. pyogenes	APAS, CARAlert

5.1.4 CARAlert

CARAlert was also established in 2016 by the Australian Commission for Quality and Safety in Healthcare for the early detection of organisms with critical antimicrobial resistance (CAR) patterns (Table 7) (24). CAR isolates are those with mechanisms or antimicrobial susceptibility patterns which pose a significant threat to the use of last-line antimicrobials (25).

Table 7. CARAlert isolates and definitions

Organism	CARAlert definition
Carbapenemase-producing Enterobacterales	Enterobacterales with confirmed carbapenemase resistance genes and/or ribosomal methylase producing species (confirmed 16s rRNA methylase genes) WGS is required to identify the full set of resistance genes (24)
Enterococcus spp.	Linezolid non-susceptible
M. tuberculosis	Multi-drug resistant (MDR) – resistant to rifampicin and isoniazid
N. gonorrhoeae	Ceftriaxone non-susceptible, and/or azithromycin resistant
Salmonella spp.	Ceftriaxone non-susceptible
Shigella spp.	MDR strains – Resistant to three or more of ampicillin, ciprofloxacin, co-trimoxazole, ceftriaxone/cefotaxime
S. aureus	Vancomycin non-susceptible or *van*A gene detected, daptomycin non-susceptible or linezolid non-susceptible
S. pyogenes	Reduced penicillin susceptibility
Additional organisms added to the CARAlert system, July 2019	
Acinetobacter baumanni complex	Carbapenemase-producing
Candida auris	All isolates
Enterobacterales	Transmissible colistin resistance
Pseudomonas aeruginosa	Carbapenemase-producing

Under the CARAlert system, all diagnostic laboratories in Australia submit suspect isolates and initial test results to a confirming laboratory which submits isolate data to the CARAlert webportal. The confirming lab is responsible for advising the referring laboratory of the results of the isolate, and the referring laboratory reports the results to the requesting doctor. In Victoria, MDU confirms the majority of Victorian CARAlert isolates including vancomycin intermediate-susceptible *S. aureus,* CPE, linezolid non-susceptible *Enterococcus* spp., and linezolid non-susceptible *S. aureus* and *N. gonorrhoea.* The Australian Mycobacterium Reference Laboratory (AMRL) at the Victorian Infectious Diseases Reference Laboratory conducts confirmatory testing for *M. tuberculosis.* There is only one other laboratory currently known to conduct any other confirmatory testing, based at Monash Health. Monash Health is only able to confirm vancomycin-intermediate susceptible *S. aureus*.

5.1.5 NEPSS

NEPSS was established in 1980 to monitor human and non-human gastrointestinal pathogens in Australia (26). While NEPSS was originally a national system, it became a mostly Victorian database in the early 2000s. NEPSS is coordinated by MDU. Any gastrointestinal pathogens can be included in the system.

Based on information from MDU Epidemiology, NEPSS is no longer actively used as a surveillance system. NEPSS currently functions as a repository for human, food, animal and environmental gastrointestinal isolates and is a longstanding database of typing and AST data. As the MDU Laboratory Information Management System (LIMS) does not currently enable searching to be performed by test result, NEPSS is used to check certain aspects of metadata associated with an isolate, for example, the specific *Salmonella* serotypes previously isolated from animals.

5.1.6 NNDSS

The NNDSS was established in 1990 by the Communicable Diseases Network Australia to coordinate national surveillance of notifiable diseases and is coordinated by the Australian Government Department of Health (27). The NNDSS compiles data on notifiable conditions from states and territories. The DHHS submits defined datasets to the NNDSS at regular intervals. At the time of writing, the only organisms with data for AMR within the NNDSS were *S. pneumoniae* (invasive disease only) and *M. tuberculosis.* In late 2019, AST data for *N. gonorrhoeae* will commence collection by the NNDSS.

5.1.7 NNN

The NNN commenced in 1979 in order to establish a standardised method of AST for *N. gonorrhoea* (28). The NNN is coordinated by the Neisseria Reference Laboratory based in New South Wales (NSW), comprising the Australian Gonococcal Surveillance Programme and the Australian Meningococcal Surveillance Programme. These programmes include monitoring of trends in AMR of *N. gonorrhoeae* and *N. meningitidis.* All Victorian AST is conducted by MDU, with data sent to the coordinating centre in NSW on a quarterly basis.

5.1.8 Victorian AMR unit

The Victorian AMR unit was established in 2019 by the DHHS in order to improve the coordination of AMR surveillance in Victoria. All notifiable diseases are reported to the DHHS by clinicians and/or laboratories. Incorporated into the surveillance of notifiable diseases, the DHHS has developed AMR surveillance for several notifiable organisms: *N. gonorrhoeae, Shigella* spp., *Salmonella* spp., *M. tuberculosis* and CPE. Surveillance of *M. tuberculosis* AMR falls under the Victorian Tuberculosis Programme and CPE surveillance is conducted as part of the Victorian CPE Surveillance and Response Unit (VCRSU). Surveillance of AMR within *N. gonorrhoeae, Shigella* spp. and *Salmonella* spp. is conducted in association with MDU.

Victorian Tuberculosis Programme

AMR surveillance is particularly important for tuberculosis (TB), as MDR isolates have high public health importance due to their need for extended treatment (up to two years), the risk of forward transmission, their association with treatment compliance issues and high treatment costs (29). Melbourne Health, based at the Peter Doherty Institute for Infection and Immunity, runs the Victorian TB programme, which has conducted TB surveillance since 2001. While

notifications are received by the DHHS, all case management, contact tracing and screening is conducted by Melbourne Health. The AMRL conducts all AST for TB.

VCRSU

CPE are bacteria of the family Enterobacterales that are identified as carrying a carbapenemase gene, which confers resistance to carbapenem antibiotics (30). The VCRSU was formed in response to a CPE outbreak in 2014 (30). The unit comprises the DHHS, MDU, and VICNISS. The aims of the unit are to detect all cases of CPE and transmission within and between healthcare facilities and to guide the management of outbreaks (30).

AMR surveillance conducted by MDU

The DHHS funds the public health laboratory work of MDU, the state's bacterial reference laboratory. Notifiable isolates are routinely sent to MDU for further typing. MDU conducts AST for selected notifiable organisms including *N. gonorrhoeae*, *Salmonella* spp., *Shigella* spp. and *N. meningitides*, and for several health-care acquired organisms including Enterobacterales, *Enterococcus* spp. and *S. aureus*. MDU provides the AST results for *Shigella* spp., *Salmonella* spp. and *N. gonorrhoeae* to the DHHS. MDU does not perform AST for all organisms it receives. For example, while MDU receives every isolate of *S. pneumoniae* for serotyping, AST is not routinely performed.

In addition to routine AST, MDU conducts cross-sectional studies of specific organisms in response to increasing numbers of cases of AMR. These snapshots are not currently performed on a scheduled basis. Previous studies by MDU have looked at CPE (2012), *Clostridium difficile* (2010 and 2012), vancomycin-resistant Enterococci (2015 and 2018) and levels of AMR within cases of invasive pneumococcal disease (2013 and 2015) (31-34).

5.1.9 VHPSS

Coordinated within MDU, the VHPSS was established in 1988 (26). The VHPSS monitors the prevalence and type of bacterial and fungal infections, and AMR, in blood and cerebrospinal fluid (CSF) specimens within Victorian healthcare facilities and the community. Data are submitted to MDU by diagnostic laboratories in one of two ways. Firstly, laboratories that have decided to actively participate in this system submit data to the VHPSS directly. Secondly, for laboratories that have not decided to actively contribute to the VHPSS, MDU extracts relevant data from LIMS where notifiable pathogens have been submitted for subtyping, and enters them into the VHPSS.

5.1.10 VICNISS

VICNISS was established in 2002 to conduct surveillance of healthcare-associated infections in order to reduce the occurrence of these infections in Victorian healthcare facilities (35). VICNISS reports on organism types and counts, including vancomycin-resistant enterococci and methicillin-resistant *S. aureus*, however does not include any AST data. The organisation plays a key role in the response to CPE in Victoria, as part of the VCRSU.

5.2 Evaluation of attributes

In this section, the attributes of surveillance in terms of overall AMR surveillance in Victoria are described. As no overall AMR surveillance system is currently in operation in Victoria, this evaluation focussed on the attributes of the combined systems that produce data on AMR. The attributes have been assessed via discussions with stakeholders and reference to available documentation. Eight diagnostic laboratories completed an online survey and an additional three participated in semi-structured interviews. Of those that completed the survey, one laboratory also participated in a semi-structured telephone interview.

5.2.1 Simplicity

Simplicity is defined as the structure and ease of operation of a surveillance system (15). The surveillance of AMR in Victoria is complex, with multiple systems involved at the state and national level. Simplicity of AMR surveillance in Victoria is therefore discussed under the following subheadings: objectives of surveillance, reporting sources and dataflow, case definitions, data submission, case follow up, and reporting.

Objectives of AMR surveillance in Victoria

The objectives of AMR surveillance vary between the surveillance systems (Table 8). For example, while AGAR has very specific objectives for monitoring trends of AMR within a subset of organisms, the Victorian AMR unit and CARAlert monitor trends but also aim to identify transmission or outbreaks of isolates with AMR. Several systems which collect AMR data do not have specific objectives in relation to the surveillance of AMR.

Table 8. Objectives of AMR surveillance systems in Victoria

Surveillance system	Objectives
AGAR	• Gram Negative Sepsis Outcome Programme: monitor AMR in Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter* spp., detect emerging resistance to last-line antimicrobials, determine the prevalence of co-resistance and multi-drug resistance, examine the molecular basis of resistance to third generation cephalosporins, quinolones and carbapenems (20) • Australian Staphylococcal Sepsis Outcome Programme: to assess the prevalence of staphylococcal isolates with methicillin resistance, in addition to assessing the molecular epidemiology of methicillin-resistant *S. aureus* (19) • Australian Enterococcal Sepsis Outcome programme: determine the proportion of *Enterococcus faecium* and *E. faecalis* bacteraemia isolates with resistance to ampicillin and glycopeptides, in addition to monitoring the molecular epidemiology of these species (36)
APAS	• To enable analysis and reporting of AMR data at a local, state and national level. This enables clinicians and policy makers to access AMR data to inform public health action
AURA	• To improve the integration of AMR data to inform control strategies at the national level (22)
CARAlert	• Early detection of organisms with critical AMR patterns (24) • To assist health departments in the identification of outbreaks of CAR isolates (25)
NEPSS	• Monitoring human and non-human gastrointestinal pathogens in Australia
NNN	• Australian Gonococcal Surveillance Programme: to monitor trends in AMR within *N. gonorrhoeae* at a national level, and to provide the data required to ensure that treatment protocols for gonorrhoea are kept up to date (37) • The objective of the Australian Meningococcal Surveillance Programme does not appear to be documented in annual reports

Victorian AMR unit	• Monitor trends in AMR • Guide response activities to prevent ongoing transmission • Detect outbreaks and monitor the impact of response measures
VHPSS	• Monitor bacterial and fungal infections and their levels of AMR in blood and CSF specimens within Victorian healthcare facilities and the community
VICNISS	• Reduction of the occurrence of health-associated infections in Victorian healthcare facilities (35)

Reporting sources and data flow

Reporting sources for AMR range from notifiable disease reports to voluntary laboratory reporting (Table 9). Unless a high proportion of laboratory participation is achieved, surveillance systems relying on voluntary reporting underestimate the true number of cases with AMR. In contrast, while reporting of notifiable diseases to the DHHS is mandatory, reports do not necessarily include data on AST.

Only two diagnostic laboratories indicated that they currently provide all AST results to the DHHS. Most laboratories provide AST results only for pathogens where the DHHS has requested them, results which vary by organism or only provide clinically relevant AST results. To achieve useful surveillance of AMR for an organism, all AST results, including both susceptible and resistant breakpoint findings and MIC values, should be obtained from diagnostic laboratories which contribute to surveillance. In addition, the guidelines used to interpret MIC values as susceptible or resistant should be obtained from diagnostic laboratories. This enables the proportion of resistance or non-susceptibility to be assessed as a proportion of isolates tested.

When asked about why they do not provide complete AST results with notifiable disease reports, most laboratories indicated they did not know that the DHHS is interested in these data. One laboratory assumed that anything sent to MDU is provided onwards to the DHHS. Several laboratories incorrectly assume that VHPSS data are forwarded onto the DHHS and the national surveillance system, AURA.

Table 9. Reporting sources of Victorian AMR surveillance systems

Surveillance system	Reporting sources in Victoria
AGAR	Five Victorian hospital laboratories — Alfred Health, Austin Health, Monash Health, Royal Children's Hospital and St Vincent's Hospital
APAS	One hospital laboratory (Monash Health)
AURA	Collates data from multiple surveillance systems (Figure 1)
CARAlert	All diagnostic laboratories refer samples to reference or confirming laboratories (MDU, AMRL, and Monash Health)
	Confirming laboratories submit data to CARAlert
NEPSS	Samples from Victorian diagnostic laboratories submitted to MDU
NNDSS	*S. pneumoniae* AST data sourced from Victorian diagnostic laboratories (not necessarily provided to the DHHS)
	M. tuberculosis AST data sourced from AMRL
NNN	MDU
Victorian AMR unit	MDU provides AST data for *Salmonella* spp., *Shigella* spp., *N. gonorrhoea* and CPE
	AMRL provides AST data for *M. tuberculosis*
VHPSS	Seven hospital laboratories, one private laboratory contribute directly
	Other notifiable isolate laboratory data is extracted from MDU LIMS
VICNISS	All public hospitals (selected private hospitals)

The current data flow for AMR surveillance is summarised in Figure 1. While the Victorian AMR unit is best placed to conduct public health follow up for AMR surveillance data, it does not currently receive all relevant data. For example, AMR data on selected pathogens causing bacteraemia are submitted by selected hospital laboratories to AGAR, and this information is not received by the DHHS. While the VHPSS is a valuable source of AMR data for several organisms, data are not currently provided to the Victorian AMR unit or forwarded onto AURA. MDU reports relevant isolates to the CARAlert system, which provides regular data summaries to the DHHS. These summaries do not include the specific healthcare origin of isolates, making it difficult for the DHHS to identify outbreaks using CARAlert data.

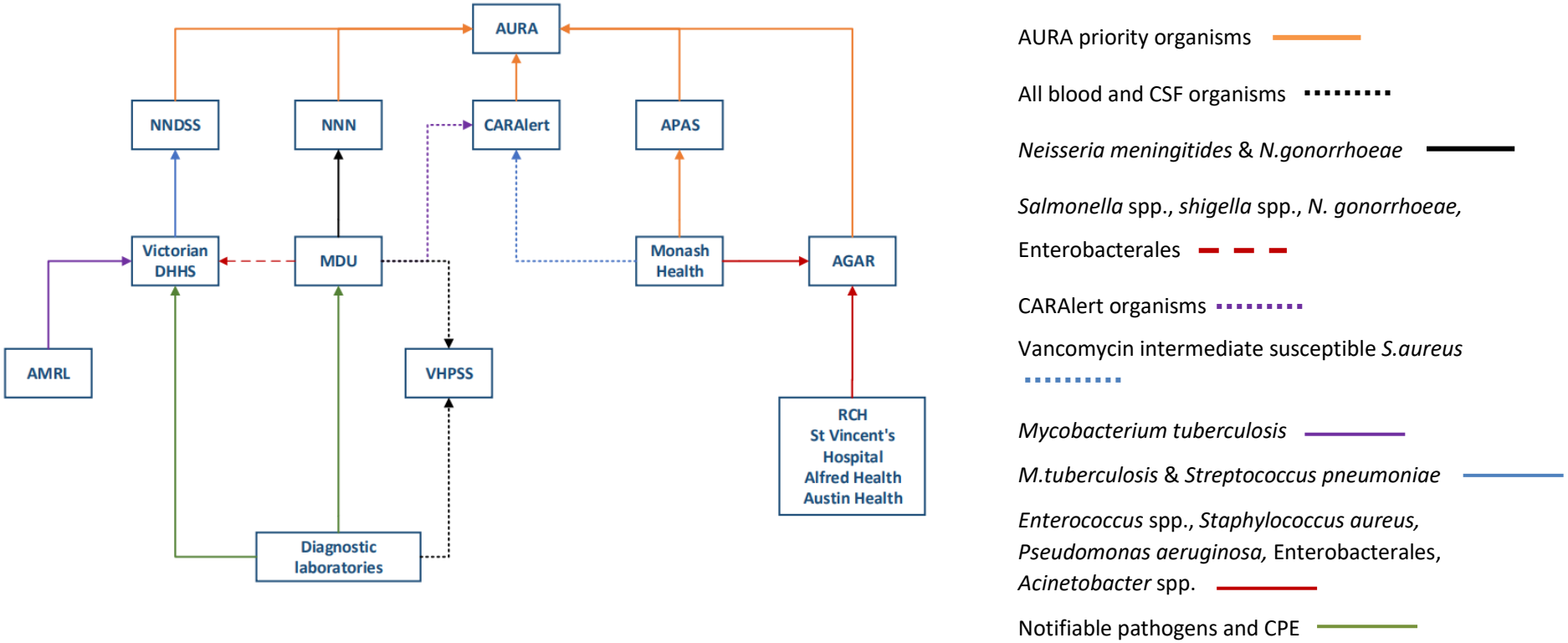

Figure 1. Pathway of AMR data movement in Victoria by reporting source and organism, 2019[4]

[4] AMRL: Australian Mycobacterial References Laboratory, AGAR: The Australian Group on Antimicrobial Resistance, APAS: Australian Passive Antimicrobial Resistance Surveillance, AURA: Antimicrobial Use and Resistance Australia, CARAlert: National Alert System for Critical Antimicrobial Resistances, MDU: Microbiological Diagnostic Unit Public Health Laboratory, NNDSS: National Notifiable Diseases Surveillance System, NNN: National Neisseria Network, RCH: Royal Children's Hospital, DHHS: Department of Health and Human Services, VHPSS: Victorian Hospital Pathogen Surveillance Scheme

Case definitions

The complexity of AMR surveillance is increased by a lack of consistency of case definitions of AMR for specific organisms between surveillance systems (Appendix 2). Systems differ in whether AMR is defined by diagnostic laboratories or reference laboratories, as well as whether MICs are interpreted as susceptible, intermediate/non-susceptible or resistant using EUCAST, CLSI, or other guidelines. While MIC values can theoretically be converted between EUCAST or CLSI guidelines, the clinical breakpoints are not consistent for all antimicrobials and therefore AST results are not necessarily comparable if interpreted using different guidelines (38-40). Possible solutions to this are that any potential differences between guidelines are evaluated for organism-antimicrobial combinations, or that all laboratories contributing to AMR surveillance for an organism use the same guidelines.

In addition, systems are inconsistent with definitions of duplicate isolates. Duplicate definitions are important as often multiple isolates are submitted for the same patient. For example, while the VCRSU includes a single isolate of CPE (for each resistant gene and subtype) for each patient, the CARAlert system uses a reinfection period of three months (24). In contrast, the VHPSS and AGAR, which also include CPE in surveillance, allow repeat isolates from patients if they have been collected more than 14 days apart.

Occasionally different AST patterns may be identified within a single patient. This is particularly relevant for AMR surveillance of *N. gonorrhoeae* as isolates with different AST may be originate from a pharyngeal and a rectal sample. In Victoria there is currently no clear or documented definition regarding which isolates should be prioritised where there are multiple isolates from the same patient. Clear case definitions for AMR will improve the simplicity and the usefulness of the surveillance of AMR within gonorrhoea. Suggested definitions for *N. gonorrhoea* are provided in Appendix 3.

Data submission

Data submission methods used by AMR surveillance systems vary. Methods include manual data entry, completion of hardcopy forms and upload of an Excel spreadsheet (Appendix 4).

Currently, different laboratory IT systems across all public hospitals are a major barrier to integrated AMR surveillance. As IT systems are not readily changeable, several laboratories expressed a preference for providing AST data extracted directly from Vitek into an Excel spreadsheet. Alternatively, several laboratories indicated that upload of a spreadsheet containing relevant data would be feasible, as this appears to be the most convenient method

of data transfer. While Vitek extracts only contain AST results, these can be linked with other notification data using the laboratory submission number.

Many diagnostic laboratories indicated that current data submission methods to various surveillance systems are challenging. I was responsible for entering CARAlert data at MDU for a large part of 2019. In my experience, manual data entry of CARAlert isolates was a time-consuming process. Systems such as AGAR collect a large amount of patient data in order to examine the factors associated with AMR, which tends to increase the burden on contributing laboratories.

Most laboratories indicated that they cannot cope with any additional workload involved in contributing to AMR surveillance, as they lack the required resources. Within this context, it is important to work with laboratories to determine the best method of data submission for AMR surveillance. The implementation of electronic submission of AMR data would assist laboratories in participating in additional surveillance activities.

The current process of manual data entry at the DHHS will be upgraded to electronic laboratory reporting, initially for four major Victorian laboratories, within the first half of 2020. This will greatly simplify the process of surveillance for AMR. Once implementation of electronic laboratory reporting is completed for the major laboratories, it will be expanded to include all diagnostic laboratories. Electronic laboratory reporting will eventually enable AST results from all laboratories to be transmitted directly into the DHHS PHESS.

Case follow up

The level of case follow-up based on AMR surveillance varies with the organism being reported. The Victorian AMR unit is currently the only AMR surveillance system able to conduct public health case follow up based on surveillance findings. Existing follow up processes are summarised in Table 10.

Table 10. Processes for the follow up of AMR cases by the Victorian AMR unit

Organism	Method of follow up	Staff responsible for case follow up	Purpose of follow up	Laboratory follow up	Data stored
CPE	Phone call	VICNISS obtains information in conjunction with healthcare facility's Infection Control Practitioner	Obtain detailed epidemiological data, including hospitalisation history, travel history, ward movements Informs epidemiological analysis to determine if case is locally acquired (as opposed to acquired overseas)	WGS on all isolates to identify resistance genes and multi-locus sequence types MDU Epidemiologists combine patient data with phylogenetic analyses to identify clusters and transmission pathways	All patient data stored in PHESS, TRA information stored on VICNISS portal (accessible by Infection Control Practitioners only)
		Doherty/DHHS Infection Control Practitioner	Works with healthcare facility per specific guidelines indicating when they are no longer affected by CPE transmission Identification of transmission risk areas (TRA); an area (usually a ward) where CPE has been known to spread between patients (30)		

M. tuberculosis	Enhanced surveillance forms are completed for all cases of TB, enabling cases to be prioritised for follow up, phone call for priority cases	Infection control/TB programme nurses at Melbourne Health	Assess risk factors for transmission (e.g. travel history), whether case is new/relapse, current treatment, treatment outcome, ensure treatment compliance, contact tracing	WGS enables identification of clusters	PHESS
N. gonorrhoea	Enhanced surveillance form sent to treating doctor (all cases), phone call for cases with critical AMR	Partner Notification Officers (specialised Public Health Officers based at the Melbourne Sexual Health Centre)	Education of cases, contact tracing	WGS on isolates with critical AMR to assist in identification of clusters	PHESS
Salmonella spp.	Phone call for cases with critical AMR	Public Health Officers	Education of cases, obtain food history, contact tracing Ensure all isolates referred to MDU	WGS on all *Salmonella* isolates as part of routine cluster identification	PHESS
Shigella spp.	Smart forms for clinical notification provides some information and all cases are contacted via a phone call	Public Health Officers	Exclude cases who work in high risk areas such as food handling and health or childcare worker Education of cases, contact tracing, ensure all isolates referred to MDU	WGS to detect transmission	PHESS

Reporting

Reports on AMR surveillance vary with each system (Table 11). While most systems produce annual reports, diagnostic laboratories indicate that more regular reporting is desirable. Some systems do not produce any regular reports on AMR surveillance data.

Table 11. Reporting outcomes of AMR surveillance systems

Surveillance system	Reporting frequency and audience
AGAR	Annual reports published online, annual meetings with contributing laboratories
APAS	Contributing laboratory can extract data and create reports, annual reports published online
AURA	Annual reports published online
CARAlert	Annual reports and bi-monthly data updates published online, fortnightly data updates are provided to state health department representatives and selected diagnostic laboratories
NEPSS	No reporting conducted
NNDSS	Annual reports including AMR data planned but several years behind
NNN	Annual reports published online
VHPSS	No regular reports, provides data upon request to contributing laboratories or the DHHS
Victorian AMR unit	Internal reports within weekly surveillance meetings, defined datasets to the NNDSS at regular intervals
	TB: internal reports within weekly surveillance meetings, quarterly reports to the TB advisory council of Victoria, International reporting to the WHO for the global TB control report, de-identified data of all confirmed cases sent to NNDSS daily
	VCRSU: Regular internal reports at DHHS surveillance meetings; MDU publishes CPE antibiograms[5] online
VICNISS	Annual reports published online, no AST data reported

[5] Antibiograms are tables of antimicrobial susceptibilities used to inform local antimicrobial prescribing

5.2.2 Data quality

Data quality is defined as the completeness and validity of the data collected by the surveillance system (15). All Australian laboratories are accredited by the National Association of Testing Authorities and operate under the Quality Assurance Program of the Royal College of Pathologists Australia (24). While data from individual laboratories are of high quality, one of the complexities with the surveillance of AMR is the collection of standardised data. To collect standardised AMR data, ideally the same AST methodology needs to be used across surveillance systems, with clinical breakpoints interpreted using the same guidelines to identify susceptible or resistant organisms. Data quality for organisms with AST conducted by MDU is high as AST values have been produced using the same testing techniques, and interpreted using the same guidelines, for organisms over time, enabling the collection of standardised AMR data (Appendix 5).

Of the 11 diagnostic laboratories that participated in this evaluation, all indicated using similar AST methodologies including Vitek (n=11), disc diffusion (n=9) and E tests (n=9). Eight of eleven laboratories indicated using CLSI guidelines exclusively for clinical breakpoint interpretation. Of the remaining three laboratories, one uses both CLSI and Calibrated Dichotomous Susceptibility guidelines, another uses both CLSI and EUCAST, and one uses EUCAST guidelines exclusively. To determine the comparability of data in terms of AMR surveillance, additional detail on the testing method for each organism under surveillance is required.

The level of standardisation of data by system is summarised in Table 12. Several systems source AMR data from diagnostic laboratories rather than reference laboratories and therefore are not able to collect standardised data as a range of AST techniques and clinical breakpoints have been used.

Table 12. Standardisation of data from AMR surveillance systems in Victoria

Surveillance system	Are AST data standardised?	Data source/issues with standardisation
AGAR	Yes	All laboratories use Vitek/Phoenix for AST, all laboratories use EUCAST guidelines to interpret clinical breakpoints
APAS	No	Any laboratory can contribute AST data
AURA	No	Sourced from AGAR, NNDSS, NNN, CARAlert, APAS
CARAlert	Yes	Only reference laboratories can confirm the identification of a CARAlert isolate
NEPSS	Yes	AST conducted by MDU
NNDSS	Yes	*M. tuberculosis*: all testing performed by AMRL
	No	*S. pneumoniae*: Victorian data derived from clinical laboratories using different testing techniques and guidelines
NNN	Yes	MDU conducts all testing using AGSP and AMSP guidelines
VHPSS	No	Many laboratories do not provide complete AST results, AST method is not always reported, and many laboratories only report a breakpoint interpretation and no MIC value
Victorian AMR unit	Dependent on organism	Data from MDU and VIDRL standardised
		Data from diagnostic laboratories not standardised
VICNISS	No AST data	Not applicable

5.2.3 Representativeness

Representativeness is defined as how accurately the occurrence of the event is described over time by the surveillance system (15). The representativeness of AMR surveillance in Victoria varies with each system and the legal requirement for data submission (Table 13).

Notifiable organisms are referred by diagnostic laboratories to MDU or AMRL for further typing including AST for some organisms. For notifiable organisms to be included in AMR surveillance, AST testing needs to be performed by the diagnostic or reference laboratory, and the results reported to the relevant surveillance system. For example, while invasive *S. pneumoniae* is notifiable, MDU does not currently perform AST for this organism. In contrast, MDU does perform AST for all *Salmonella*, *Shigella* and *N. gonorrhoea* isolates, and as the DHHS has requested that all AST results for these organisms are forwarded to the Epidemiologist for AMR, these organisms are well represented in surveillance conducted by the Victorian AMR unit.

Non-notifiable healthcare acquired organisms that are not included in the CARAlert system are not well-represented in AMR surveillance in Victoria. AMR surveillance for these organisms is also affected by the specific AMR case definitions of each system conducting surveillance. For example, AGAR conducts sentinel surveillance of all bacteraemia isolates of vancomycin-resistance *Enterococcus* spp., but only monitors a specific number of antimicrobials.

Table 13. Summary of representativeness of AMR surveillance in Victoria

Surveillance system	Mandatory or voluntary submission	Specimen types	Number of laboratories contributing
AGAR	Voluntary	Blood only	Five large hospital-based laboratories only No private laboratories
APAS	Voluntary	Any	Only one Victorian laboratory based at a large urban hospital currently contributes
AURA	Voluntary	Per individual systems	Varies with species Laboratories contributing to AGAR, CARAlert, NNDSS, NNN
CARAlert	Voluntary	Per case definitions	All
NEPSS	Voluntary	Any	Unknown
NNDSS	Mandatory	All cases that meet the case definition for	All

		notifiable diseases are reported to the NNDSS	
NNN	Voluntary	Per case definitions	All
Victorian AMR unit	Mandatory for notifiable diseases, CPE and *Candida auris*	All per case definitions	All
VHPSS	Voluntary	Blood and CSF only	• Seven laboratories contribute directly to the VHPSS • For notifiable pathogens referred to MDU for typing or other testing, relevant data will be extracted from LIMs and entered into the VHPSS. • Non-notifiable pathogens will not necessarily be referred to MDU and are therefore only included in VHPSS if contributing laboratory provides data
VICNISS	Depends on surveillance type[6]	Any	No AST data collected

[6] All health services must participate in *S. aureus* bacteraemia and *Clostridium difficile* surveillance under the *Victorian Public Health and Wellbeing Act 2008*

The representativeness of AMR surveillance is affected by several other factors which vary with the specific organism (Figure 2). For example, the representativeness of CPE surveillance is affected by the testing and screening practices of clinicians and healthcare facilities. Screening protocols are not standardised in Victoria, and as CPE may colonise a patient whilst causing no symptoms of illness, whether a screening test is performed will determine if a CPE case is reported to the VCRSU.

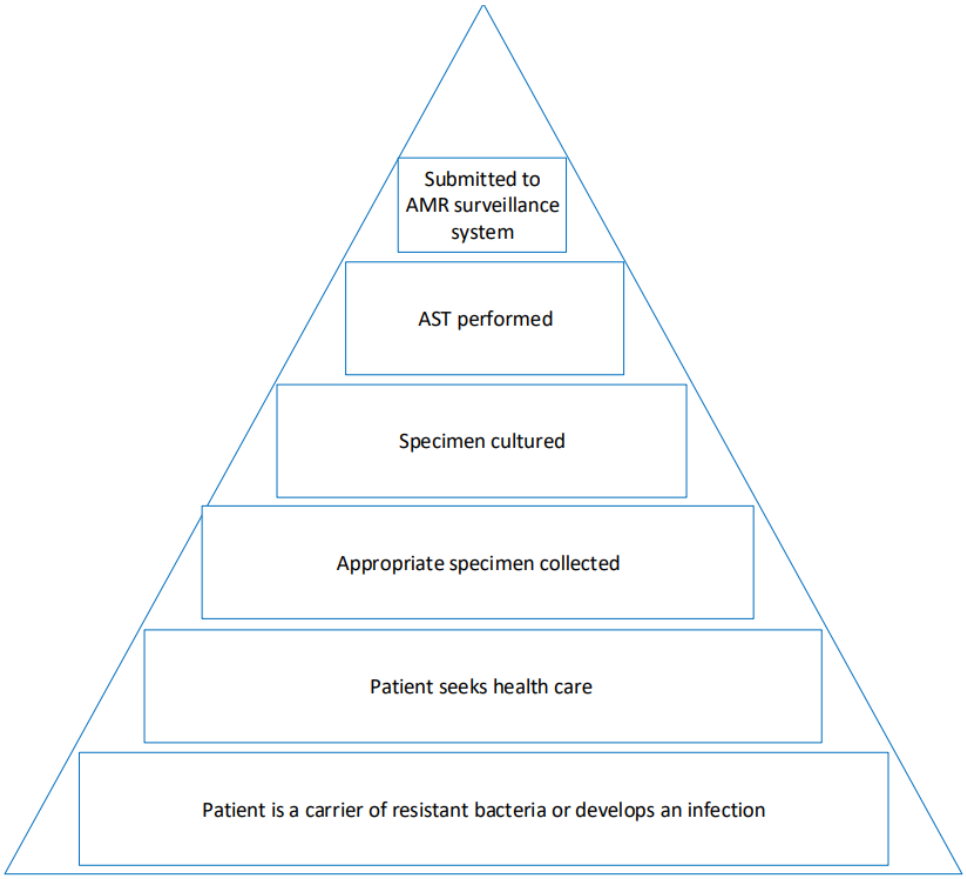

Figure 2. Surveillance pyramid for AMR surveillance

Another factor in the representativeness of AMR surveillance is whether an appropriate test is ordered on an appropriate specimen to enable AST. For example, phenotypic AST cannot be performed with nucleic acid amplification tests (NAAT) which may be used for diagnosis of bacterial pathogens. This is particularly relevant to *N. gonorrhoea.* In 2017, *N. gonorrhoea* isolates with AST performed represented only 31% of gonorrhoea cases notified in Victoria, as the majority of cases were diagnosed with NAATs (41). Previously, laboratories were only funded to perform a single diagnostic test to diagnose an organism. Consequently, many clinicians only requested NAAT. As of 2019, the implementation of reflexive culture under the Medicare

Benefits Schedule is expected to increase the representativeness of AST surveillance for notifiable pathogens, as laboratories will be funded to perform culture and sensitivity if these pathogens are confirmed by NAAT. This process will reduce the number of cases diagnosed only by NAAT and therefore increase the number of isolates able to be tested for phenotypic antimicrobial susceptibility.

Within Victoria, the only laboratories contributing to AGAR are based within hospitals, and no private laboratories contribute. Therefore, AMR within the community is not well represented by AGAR. As a relatively small number of laboratories contribute to AGAR, which feeds data into the AURA system, Victorian AMR data, particularly data on AMR within the community are also underrepresented within the AURA system. Representativeness of Victoria AMR data could be improved by contributing to AURA via an existing surveillance system such as the VHPSS, or by providing additional AST data from MDU, with the consent of diagnostic laboratories.

5.2.4 Timeliness

Overall, AMR surveillance in Victoria lacks the timeliness required to effectively respond to emerging AMR and outbreaks. For example, for systems based within Victoria, the time between sample submission to MDU and reporting of AMR data to the DHHS ranges from two weeks to three months, depending on the organism (Appendix 5.). In order to identify and respond to cases of concern, optimal timeliness for an AMR surveillance system is real-time surveillance. Timeliness is affected by multiple factors including the time taken for samples to be submitted to the laboratory, the time taken for culture and AST to be completed, data entry, analysis, reporting and case follow-up. While real-time surveillance may not be achievable in the near future, the frequency of data provision and reporting needs to be increased.

5.2.5 Acceptability

Acceptability is the willingness of organisations to participate in the surveillance system (15).
The current structure of AMR surveillance in Victoria does not appear to be acceptable for most diagnostic laboratories. As the surveillance of AMR is largely laboratory-based, a large part of the work burden falls on laboratory staff.

Many laboratories reported that participation in AMR surveillance is time-consuming and resource intensive. Reported reasons for not participating in AMR surveillance were largely a lack of resources and time, represented by the following comment:

> *Realistic funding (is required) as scientists need to be taken off usual tasks to perform data collection, entry, and isolate retrieval. This is a significant opportunity cost. This is a luxury we are struggling to meet.*

In addition, several laboratories expressed concern about duplication of effort and additional AMR surveillance, represented by the following two comments:

> *Currently (it) seems that there is duplication in that there are too many systems – it would be better to have one centralised system (ideally a national one). Multiple systems create an increased work burden for laboratories.*

> *(There is) concern about increasing the complexity of AMR surveillance would lead to strained relationships and reduced compliance of laboratories contributing data.*

To minimise duplication and additional workload for laboratories, it is important to define priorities for AMR surveillance, in terms of specific organism antimicrobial combinations and objectives. These priorities should be set based on consultations with laboratories and clinicians. Diagnostic laboratories were asked to comment on what other organisms should be prioritised for AMR surveillance, in addition to those already under surveillance. Many laboratories indicated organisms that are not currently a focus of routine AMR programs. These include *Pseudomonas* spp., *Acinetobacter* spp., *Clostridium difficile,* vancomycin-resistant Enterococci, quinolone and gentamicin resistance in Enterobacterales, and Extended Spectrum Beta-Lactamases.

In addition to developing surveillance priorities, surveillance activities should avoid creating unnecessary work for laboratories; contribution to surveillance should be as simple as possible. The minimum amount of required data should be requested, data submission should be simple, and data needs to be disseminated to relevant parties in a timely manner (42).

In order to complete the cycle of surveillance, it is essential that regular feedback on AMR surveillance is provided. While laboratory liaison meetings, which both the DHHS and representatives from all diagnostic laboratories attend, are currently scheduled biannually, several AMR surveillance systems do not provide contributing laboratories with regular reports. When asked about future AMR surveillance and preferences for receiving reports, most diagnostic laboratories prefer the circulation of surveillance reports via email rather than reports being published online. One laboratory specifically requested that reports are sent as a link via email, rather than as an attachment. Most laboratories prefer quarterly reports, in addition to annual reports.

5.2.6 Usefulness

A useful surveillance system is one which contributes to the prevention and control of AMR (15). A useful AMR surveillance system should be able to identify outbreaks or resistant organisms and identify emerging resistances. In addition, AMR surveillance systems should provide the data on AMR surveillance to end users such as diagnostic laboratories, healthcare facilities and clinicians, in order to inform antimicrobial stewardship and treatment guidelines (42). A useful AMR surveillance system should also prioritise pathogens for surveillance and use the surveillance data to inform appropriate public health action (42). To meet this requirement, ideally AMR surveillance needs to be combined with data on antimicrobial use.

Current surveillance activities in Victoria are useful for monitoring long-term trends. The usefulness of individual AMR surveillance systems is discussed below.

Victorian AMR unit

The Victorian AMR unit receives AST for a limited subset of organisms. For the organisms where AST data are received, public health officers and partner notification officers are able to contribute to the control of AMR by:

- Liaising with laboratories and doctors to ensure that isolates are sent to MDU for typing and AST
- For isolates with AMR, arranging clearance testing and/or referral to an infectious diseases specialist
- Conducting contact tracing and follow up testing as required

While case follow-up is effective in preventing the spread of AMR to additional cases, it does not address the underlying factors involved in AMR. For example, an increase in the number of gonorrhoea cases with AMR may relate to previous antimicrobial use in the patients. Systems such as the as the Gonococcal Isolate Surveillance Project, operated by the Centers for Disease Control and Prevention, and the European Gonococcal Antimicrobial Surveillance Programme, operated by the European Centre for Disease Prevention and Control, collect extensive variables in order to understand the factors associated with AMR (Appendix 7).

The Victorian TB programme is highly useful due to the level of treatment supervision and follow-up provided. Follow-up enables education of cases and improves treatment compliance. Melbourne Health conducts case follow up based on risk level, with pulmonary cases being prioritised due their infectious nature. Patients with pulmonary TB are isolated until they have been treated for 14 days and obtained a negative sputum smear. All patients with MDR TB receive directly observed therapy in order to increase the likelihood of successful treatment

completion. Contact tracing is also prioritised by risk and minimises the possibility of further transmission of TB.

The VCSRU is useful for monitoring trends of CPE, detecting and managing outbreaks and transmission within and between healthcare facilities. In response to the identification of locally acquired cases, control measures are implemented to prevent more patients becoming infected. CPE antibiograms created by MDU are useful for clinicians however they are currently published on the MDU website and are not circulated directly to relevant clinical staff. As the aim of antibiograms is to assist clinicians who treat patients with suspected CPE infections, distributing this data directly to healthcare facilities is expected to improve the usefulness of the VCRSU.

AURA and CARAlert

AMR surveillance conducted by AURA and CARAlert is successful at monitoring long-term trends. Laboratories based within healthcare facilities indicate that they use AURA and CARAlert data to understand local AMR epidemiology, which inform local infection control procedures:

> *It is good to know local epidemiology on selected bacterial antimicrobial resistance……to be kept posted about emerging resistance in our region. We can share this with hospital infection control, infectious diseases physicians and hospital clinicians…… This may encourage increase(d) vigilance and focus on promoting hand hygiene, cleaning and contact precaution for managing patients with enteric symptoms.*
>
> *Understanding of local epidemiology assists in interpreting local susceptibility trends, as well as providing an overview of broader susceptibility patterns nationally.*

In addition, CARAlert reports are used by MDU to compare AMR in Victoria to other states. CARAlert data are presented at monthly MDU laboratory meetings in order to inform laboratory testing practices.

AGAR

Laboratories participating in the AGAR scheme tend to use the data to inform internal antimicrobial stewardship programmes:

> *Education and improving local knowledge of the epidemiology of AMR, and providing a standard for comparison of resistance genes/phenotypes in the laboratory*
>
> *Used to understand what MRSA and VRE genes and clones are circulating in Australia….and comparing our hospital to the state….this identifies the main multi-*

> *resistant bacteria…to focus on at our institution….which allows for screening of targeted units and enhancing cleaning methods and hand hygiene*

AGAR surveillance data are also useful for comparison to European data, as this programme follows the methodology used by the European Antimicrobial Resistance Surveillance Network. One stakeholder commented that AGAR needs to develop a more specific research question relating to the collection of clinical data, such as thirty-day mortality, in order to enhance the use of the data. While AGAR annual reports include information on the subtyping, phylogenetic analyses are only conducted as part of research and these results are not fed back to contributing laboratories.

Data on antimicrobial use

While current AMR surveillance activities successfully monitor trends of AMR, surveillance data are only combined with data on antimicrobial use at the national level. To improve the usefulness of surveillance conducted within Victoria, data on AMR should be combined with data on antimicrobial use at the local level. This could be achieved by forming collaboration with the National Centre for Antimicrobial Stewardship, based in Victoria, which conducts research in order to improve antimicrobial prescribing in Australia. Reporting AMR data together with data on antimicrobial use would lead to improved understanding of factors involved in the emergence of AMR (43).

Reporting

The current usefulness of AMR surveillance in Victoria is limited by the lack of timely, consistent dissemination of data to end-users. Implementing regular, timely reports on AMR surveillance is essential to complete the surveillance cycle and achieve useful surveillance. Increasing the frequency of reports would also enhance the acceptability of the surveillance activities. This is particularly important for diagnostic laboratories who feel increasing burden from the workload associated with the provision of AMR data to multiple systems.

Representativeness

The usefulness of AMR surveillance in Victoria is also affected by the representativeness of the systems, many of which have low levels of representativeness due to the voluntary nature of data contribution. AURA and AGAR notably have low levels of contribution by Victorian organisations, meaning it is difficult to accurately compare Victorian data to other jurisdictions.

Surveillance within the context of existing activities

Currently, the DHHS largely conducts AMR surveillance for organisms tested by MDU only, as complete AST data are not routinely provided by diagnostic laboratories. While the usefulness of AMR surveillance would be improved by additional laboratories participating in surveillance, some laboratories expressed concerns about the need to provide additional data without a clear objective:

> *Need to be careful about asking laboratories to contribute more data without having a clear objective as to why, and being clear about what data, and for what purpose.*
>
> *The number of institutions requesting more data transfers and isolates to be sent for further analysis, without any financial support to be compensated for this extra work, needs to be understood and considered, so only the most important things will be prioritised.*

These comments highlight the importance of implementing AMR surveillance within the context of existing surveillance activities.

5.2.7 Resource requirements for AMR surveillance

The resources currently required for AMR surveillance in Victoria are summarised in Table 14. It is evident that significant resources are already being used in contributing to existing surveillance systems. As there are limited additional resources currently available, it is important to prioritise surveillance activities and to carefully consider how collected data will be used to inform and implement public health action.

Table 14. Victorian resources used in AMR surveillance

Surveillance System	Resources
AGAR	• Laboratory scientist combines AST data with patient data and uploads AST data to webportal • Medical microbiology registrar collates patient metadata • Packaging and sending isolates interstate for sequencing (Western Australia/South Australia) • Sample storage prior to submission, packaging of isolates to send away for typing • Staff time to contribute data; 10 to 20 hours per month on data extraction and collection of patient metadata
APAS	• Requires significant IT infrastructure to participate in system
AURA	• No additional resources to contribute currently
CARAlert	• Diagnostic laboratories submit relevant isolates to MDU/AMRL • Manual data entry to CARAlert webportal, conducted by MDU • MDU conducts confirmatory testing of isolates • IT infrastructure at MDU to filter relevant reports
NEPSS	• Extraction of MDU LIMS data and upload into NEPSS
NNDSS	• Data submission to NNDSS by DHHS
NNN	• Data submission to coordinating centre by MDU
VHPSS	• Data requires checking by data manager and epidemiologists to ensure case definition is met • Full time data manager follows up missing data variables • Epidemiologist
Victorian AMR Unit	• Policy advisor • Epidemiologist • Biostatistician • Public Health Officer • VCRSU epidemiologist (based at MDU) • TB epidemiologist (component of work role) • Melbourne Health Infection control nurses • Data submitted to DHHS by MDU • DHHS funds AST conducted by MDU for notifiable organisms
VICNISS	• Epidemiologist

6. Discussion

This evaluation has described the AMR surveillance activities conducted for human isolates in Victoria. Several issues were identified and recommendations made.

AMR surveillance activities in Victoria lack cohesion. This can be greatly improved by building on existing AMR surveillance conducted by the Victorian AMR unit. Once the Victorian AMR unit is fully operational, it is expected that it can act as a central coordinating body for Victoria. This would improve the simplicity of AMR surveillance in Victoria. A single coordinating entity is also likely to reduce duplication of effort for AMR surveillance in Victoria.

While the timeliness of AMR surveillance could be improved by significant upgrades of information technology systems, this is an expensive and time-consuming solution. Rather, timeliness is expected to improve as a result of implementing clear governance, potentially through the further development of the Victorian AMR unit.

6.1 Objectives of AMR surveillance

To improve the usefulness of future AMR surveillance, it is essential that objectives are clearly defined and documented. Current objectives of the Victorian AMR unit are to monitor trends of AMR among key organisms and identify and respond to outbreaks of resistant organisms. For collected surveillance data to inform public health action, additional proposed objectives could be:

- To collect and disseminate data on AMR, in a timely manner, to clinicians and healthcare facilities in order to inform antimicrobial stewardship and prescribing
- To combine data on AMR with data on antimicrobial use at the jurisdictional level, in order to inform required changes in antimicrobial stewardship
- To gain an understanding of the factors associated with the emergence of AMR

6.2 Resource constraints and prioritisation of surveillance activities

Resource constraints were a common theme throughout this evaluation. Diagnostic laboratories lack the resources to take on any additional work required to participate in surveillance. In addition, variation in laboratory information management systems do not enable AST data to be readily shared. Of concern, many surveillance activities are currently performed without dissemination of data in the form of reports. Given the resource-constrained setting, it is important to prioritise future surveillance activities for AMR. This could be achieved by

development of an AMR strategy for Victoria, developed in conjunction with key stakeholders such as healthcare facilities, diagnostic laboratories, and coordinators of existing AMR surveillance systems.

From the perspective of the DHHS and the AMR unit, it is most feasible to focus on developing and enhancing surveillance of AMR for notifiable organisms. As demonstrated by the surveillance and response of CPE, surveillance of healthcare acquired infections is complex, and requires the participation of multiple organisations and the collection of detailed patient data in order to achieve a useful public health response. Currently, the Victorian AMR unit has well developed surveillance processes for a small number of notifiable organisms. Once surveillance activities are well established for notifiable organisms, potentially the same model can be extended to additional pathogens.

In determining which organisms to prioritise for AMR surveillance, it is essential to consider the existing surveillance activities described throughout this evaluation. Focusing on organisms already being tested for antimicrobial susceptibility, where data are not already being provided to the DHHS, may initially be the most efficient way to conduct additional AMR surveillance. For example, *S. pneumoniae* is categorised as an urgent AMR threat by the Centers for Disease Control and Prevention and is routinely tested for antimicrobial susceptibility by diagnostic laboratories. AST data for invasive isolates are included in the VHPSS and could feasibly be provided to the DHHS on a regular basis.

Of note, the VHPSS appears to be an underused data source. Many laboratories were under the impression that VHPSS data are already being provided to the DHHS on a regular basis. This misunderstanding further highlights the importance of regular reporting to stakeholders as part of surveillance.

Consideration of resourcing additional AMR surveillance is demonstrated by evaluating the implementation of *S. pneumoniae* and vancomycin-resistant *E. faecium* (VREfm) surveillance. To conduct AMR surveillance for invasive pneumococcal disease, the DHHS would need to consider whether data from the VHPSS (originating from diagnostic laboratories) or data from MDU are required. To maintain the acceptability of AMR surveillance in Victoria, it is preferable to use VHPSS data rather than requesting laboratories submit duplicate data to the DHHS. In order to conduct surveillance using VHPSS data, the system would need to implement the collection of AST methodology used by each laboratory, in addition to clinical breakpoints used, and MIC values. Alternatively, to collect standardised AST data, the DHHS would need to fund MDU to conduct AST testing for all invasive *S. pneumoniae* isolates. Similar considerations would apply for the surveillance of AMR among other notifiable organisms.

In Chapter IV I describe a month-long surveillance study of the genomics of VREfm in Victoria. While it has been proposed that only one genotype of VREfm, *van* A, will be notifiable in Victoria as of 2020, it is expected that surveillance and response will most likely follow a similar process to that used for CPE. This would result in increased workload for MDU Epidemiology, who are also responsible for managing the VHPSS. Furthermore, as VREfm bacteraemia are already under surveillance by AGAR, with isolates sent interstate for sequencing, surveillance would require collaboration with interstate laboratories.

Comprehensive continuous surveillance is appropriate for organisms where AMR is known to be a current threat, however for other organisms it may be more appropriate to conduct intermittent or sentinel surveillance (42). For each organism under surveillance, given the available resources, it needs to be decided whether continuous surveillance is essential, or if another approach such as 'snapshots' or cross-sectional studies conducted at regular intervals are more appropriate. In response to a snapshot that identifies an issue of emerging surveillance, continuous surveillance practices could then be implemented.

Considering the above factors, it appears that the least resource intensive organism to add to surveillance would be one already included in the VHPSS, such as *S. pneumoniae.* However, due to the previously described issues with current surveillance processes, it may be more successful to focus on surveillance and reporting for a limited number of pathogens, and then expand the number of organisms once systems are more timely, acceptable and useful.

While it is important to work towards a one health approach to the surveillance of AMR in Victoria, clear processes which include a dissemination plan for surveillance data must be implemented before surveillance activities are expanded to included veterinary, food, and environmental data sources.

7. Recommendations for AMR surveillance in Victoria

Based on the attributes of current AMR surveillance, the follow recommendations have been made:

Objectives and coordination of AMR surveillance

- Clarify the primary objective(s) of AMR surveillance in Victoria, including clearly defining which organism-antimicrobial combinations are the focus of surveillance.
- Define and document which data variables are required to appropriately monitor and respond to increasing levels of AMR.

- Determine for each organism-antimicrobial combination whether continuous surveillance or snapshot surveillance at defined intervals is the best method given available resources.
- Together with diagnostic laboratories and coordinators of AMR surveillance systems, develop a Victorian AMR strategy to assign and document responsibilities for each aspect of surveillance.

Usefulness of AMR surveillance

- Where possible, work to harmonise organism-specific case definitions of AMR between surveillance systems.
- Implement regular reporting on CPE surveillance and response.
- Continue developing and publishing CPE antibiograms and ensure clinicians are aware of antibiograms by circulating to relevant networks.
- In consultation with diagnostic laboratories, implement regular provision of VHPSS data, for specific organism-antimicrobial combinations, to the DHHS and/or AURA.
- For data that cannot be sourced from the VHPSS, request that all diagnostic laboratories submit AST data as defined by organism-antimicrobial combinations prioritised for surveillance within the DHHS.
- In consultation with diagnostic laboratories, consider reporting of CARAlert data from MDU to the DHHS.
- Request that MDU provides AST data for all priority pathogens to the DHHS on a more regular basis, at least monthly.
- To understand the factors associated with emerging AMR, consider the collection of additional data variables for pathogens where the DHHS is conducting AMR surveillance.

Data quality of AMR surveillance

- As diagnostic laboratories use varying guidelines to interpret clinical breakpoints, work with laboratories to implement a method of collecting the guidelines used. As guidelines used may change, these should be included on all AMR data submissions.
- Modify the VHPSS submission form to include the AST method used and the clinical guidelines used to interpret antimicrobial susceptibility results.

Acceptability of AMR surveillance

- Increase the frequency of communication with diagnostic laboratories. This could be achieved by increasing the frequency of Laboratory Liaison meetings to enable clear discussions of planned changes to AMR surveillance in Victoria.
- Work with diagnostic laboratories to determine the best method of data submission for AMR surveillance.
- Once AMR surveillance is implemented within the DHHS, work towards the distribution of quarterly data updates and annual reports to laboratories via email.
- Reinstate regular reporting by the VHPSS, either directly by the VHPSS or as part of DHHS AMR surveillance reports (if VHPSS data are provided to the DHHS).
- Ensure that CARAlert data updates are received by all laboratories who contribute isolates for testing

Enhancing AMR surveillance in Victoria

It appears that there are three main areas for improvement:

- **Improving coordination and governance to ensure that AMR surveillance findings are disseminated to laboratories, clinicians, and healthcare facilities in order to maximise public health impact.** This can be achieved through centralised coordination of surveillance activities. Potentially, the development of a Victorian AMR strategy which documents organism-antimicrobial priorities, surveillance processes and standards, a data dictionary and dissemination plan.
- **Improve the use of existing surveillance data.** This can be achieved most readily by ensuring VHPSS data are provided to the DHHS on a regular basis, and potentially provided onwards to the national AURA system.
- **Work towards collaboration with organisations conducting surveillance of antimicrobial use.** This will ensure that collected AMR data is used to inform antimicrobial stewardship.

8. Conclusions

Current surveillance activities in Victoria are successful in monitoring long-term trends of AMR. Improvements in dissemination of surveillance data and the timeliness of data sharing and reporting will greatly improve the public health impact of AMR surveillance in Victoria.

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Appendix 1. Antimicrobial susceptibility testing techniques

Broth micro-dilution (34)

Broth micro-dilution is the reference method for AST and is performed on a large scale with machinery such as the Vitek 2® (Biomerieux) and the Sensititre® (Thermo Fisher Scientific). The Vitek 2® uses cartridges that contain antimicrobials which are incubated with a standardised bacterial suspension of the organism in question. This produces a growth curve for the particular organism, which is then compared to a standard curve. This machinery is used in most diagnostic laboratories and produces quantitative results (MIC) in addition to qualitative results (susceptible, intermediate or resistant). The Sensititre® uses antimicrobials which are freeze-dried in 96-well plates, enabling many samples to be tested at once.

Disc diffusion

Using broth-microdilution methods, most organisms are able to be tested for AST however certain fastidious organisms require different techniques such as disc diffusion (34). This technique involves the use of paper disks containing antimicrobials placed on agar plates containing the organism being tested (44). AST is then performed by measuring the size of the area of inhibition of bacterial growth around the disks after a designated time in an incubator at a standardised temperature (45).

Agar dilution

This technique involves placing bacteria onto agar plates containing different concentrations of antimicrobials (45). The AST result is determined by the highest concentration of antimicrobial that inhibits growth for that organism. This is more labour-intensive than automated methods, however may be used for bacteria with a rapid growth rate (45).

E-test (antimicrobial gradient assays) (34)

The E-test uses a strip which is impregnated with a gradient of concentrations of an antimicrobial. A standard inoculum of organism is spread across a standard agar plate and the E-test strip is then applied to the agar plate. The MIC result is read directly from the strip where the organism first touches it. This test was originally designed for more fastidious organisms. It is also useful for testing antimicrobials not included in the pre-designed freeze-dried panels used in the Sensititre®.

Appendix 2. Case definitions for AMR surveillance[7]

Surveillance system	Case definition for inclusion in the system	Definition of duplicates	Case definition for AMR
AGAR	Bloodstream isolates only: *E. faecium* and *E. faecalis*, *S. aureus*, Enterobacterales, *Acinetobacter* spp. and *P. aeruginosa*	Repeat isolates from a patient are included if they have been collected more than 14 days after the first positive specimen.	As per EUCAST guidelines
APAS	All isolates	Not defined	Antimicrobial susceptibility as interpreted by diagnostic laboratories
AURA	Specific for each organism	As determined by originating surveillance system	As determined by originating surveillance system
CARAlert	Specific for each organism, must be notified by a confirming laboratory	CARAlert isolates collected on the same day or in the same admission period, or within three months (24) however MDU does not receive data on admission dates Isolates are entered using the MDU laboratory identification number; no unique patient identifier is used. A single patient may have several isolates submitted under multiple different MDU laboratory	As specified in section 5.1.4

[7] VICNISS is not included in this table as it does not include AST in surveillance

		identification numbers; therefore, the web portal may not enable accurate identification of duplicates. No documented method of data entry where a patient has dual isolates originating from different specimen types.	
NEPSS	Enteric pathogens which arrive at MDU for further typing	Not documented	Antimicrobial susceptibility as interpreted by diagnostic laboratories
NNDSS	Invasive pneumococcal disease: isolation of *S. pneumoniae* from a normally sterile site by culture or by NAAT	*S. pneumoniae*: 30 days	AST data for *S. pneumoniae* originate from primary diagnostic laboratories in Victoria - needs to be culture positive to have an AST
	M. tuberculosis: confirmed cases are those diagnosed through isolation of the organism by culture or NAAT (except where there is a possibility of inactive or previous disease), or those diagnosed clinically. Only active TB infections are notifiable, latent infections are not notifiable.	*M. tuberculosis:* not applicable	As defined by Victorian TB programme

NNN	Australian Gonococcal Surveillance Programme (AGSP): any sample from which *N. gonorrhoea* is isolated	*N. gonorrhoea* – 21 days	Using AGSP guidelines
	Australian Meningococcal Surveillance Programme (AMSP): any sample of *N. meningitidis* considered invasive (blood, cerebrospinal or joint fluid, or a positive nuclear acid amplification test from a normally sterile site) (46).	*N. meningitidis* – 30 days	Using AMSP guidelines
VHPSS	All invasive isolates (blood and cerebrospinal fluid samples), any species	Repeat isolates from a patient are included if they have been collected more than 14 days after the first positive specimen	Antimicrobial susceptibility as interpreted by diagnostic laboratories
Victorian AMR unit	All notifiable diseases are reported to the DHHS	Gonorrhoea notifications are considered to be re-infection if occurring 3 or more weeks after the initial notification	High level resistance to azithromycin (MIC ≥256mg/L) and/or Ceftriaxone resistance (MIC>0.125mg/L)
		Shigellosis 6 months	• MDR as per CARAlert case definitions • Reduced susceptibility to ciprofloxacin (MIC ≥1mg/L and resistance to azithromycin (MIC ≥16mg/L)

		Salmonella 6 months	MDR (resistant to 3 or more antimicrobial classes)
		TB – not applicable	• MDR TB: resistant to isoniazid and rifampicin • Presumptive MDR TB includes any cases with a GeneXpert test indicative of rifampicin resistance (a PCR test which assesses rifampicin resistance in addition to the presence of *M. tuberculosis)* • Extremely drug resistant TB: MDR plus resistance to one of ciprofloxacin, moxifloxacin, ofloxacin, norfloxacin, levofloxacin plus resistant to one second line drug (amikacin, kanamycin or capremycin)
	VCRSU: Confirmed cases of CPE include both clinical infections and colonised patients, however must have a carbapenemase gene detected by MDU (30)	• Once infected or colonised by CPE, a patient is considered to be a case of CPE indefinitely, as the duration of colonisation is currently unknown. • Isolates of a different species, resistance gene or multi-locus sequence type are considered to be an additional episode	• Suspect CPE include any Enterobacterales isolate with a meropenem minimum inhibitory concentration of or more than 0.5mg/L (30).

Appendix 3. Definitions for AMR surveillance of gonorrhoea used by international systems

It is thought that cephalosporin resistance emerged by commensal *Neisseria* species in the pharynx interacting with *N. gonorrhoea* present in the pharynx. As cephalosporins and other antimicrobials are often poorly effective in the pharynx, pharyngeal samples should have first priority as cephalosporin resistance is most likely to develop in the pharynx (47).

Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP), Public Health England (48)

Where there is more than one isolate per patient with different AST profiles, the more resistant code is prioritised for surveillance. If there is more than one isolate collected from multiple specimens for the same patient, isolates are prioritised using the following hierarchy:

- Males: rectal, urethral
- Females: cervical, any other site

European Gonococcal Antimicrobial Surveillance Programme, European Centre for Disease Prevention and Control (EUROGRASP) (47)

Where there is more than one anatomical site infected in one patient, only one isolate is included in surveillance using the following hierarchy:

- Males: pharyngeal, rectal, urethral, other
- Females: pharyngeal, cervical, other anogenital (high vaginal swab, rectal, urethral), other

Appendix 4. Data submission methods for selected AMR surveillance systems in Victoria

Surveillance System	Submission method
AGAR	• submitted either by manual entry in a webportal, or upload of an Excel spreadsheet to the portal
APAS	• APAS uses a data cube which accepts data extractions from laboratory information systems
CARAlert	• Diagnostic laboratories submit isolate with hardcopy CARAlert form or routine laboratory reports to MDU • MDU manually enters data into CARAlert portal • Relevant lab reports are manually checked to ensure they meet the CARAlert submission criteria prior to entry • Selected data points require verification via the original laboratory submission form via the laboratory information management system • MDU conducts WGS of all CPE isolates in order to identify resistance genes and confirm the identity of the isolate as CPE. As CPE subtypes are derived from WGS data, they are entered several days after initial confirmation results. At times, an isolate initially entered as CPE is later revealed to be both a ribosomal methylase and CPE isolate. The current system does not allow these data to be updated by the person entering data and this information is emailed to the CARAlert coordinator for updating
NNDSS	• DHHS sends relevant AST data to NNDSS quarterly for *S. pneumoniae* and daily for *M. tuberculosis*
NNN	• MDU sends AST data in Excel spreadsheet on a quarterly basis

VHPSS	• Completion of hard copy form, submission of a Vitek printout or routine laboratory report • Submitted by mail or fax • Data entered into VHPSS manually via a portal
Victorian AMR unit	• All notifiable disease reports from diagnostic laboratories and doctors are received by the DHHS via fax, email or hardcopy forms • Data are then manually entered into the Public Health Event Surveillance System (PHESS) • AST data received from MDU via emailed password-protected Excel spreadsheet • Currently, AMRL notifies the DHHS of all TB cases by fax or hard copy form. • Notifications are checked by the TB epidemiologist each day and couriered to the TB programme each afternoon. If MDR TB is notified to the DHHS, the TB epidemiologist contacts Melbourne Health by phone call • As with other aspects of AMR surveillance, implementation of electronic laboratory reporting will improve the notification process, enabling TB notifications to be transferred to Melbourne Health via password protected pdf documents • Diagnostic laboratories submit suspected CPE to MDU using the CPE isolate referral form, which collects patient demographics in addition to details of hospital admissions and movements. All data stored in PHESS – AMR data are uploaded from excel spreadsheets

Appendix 5. AST conducted by MDU

Organism	Testing technique	Guidelines used
***Enterococcus* spp.**	Sensititre broth microdilution	EUCAST
Enterobacterales	Vitek, Sensititre broth microdilution E tests	EUCAST with CLSI where EUCAST breakpoints not available
N. gonorrhoeae	Agar dilution E tests	AGSP
N. meningitides	Agar dilution E tests	AMSP
***Salmonella* spp.** ***Shigella* spp.**	Agar dilution E tests	CLSI and EUCAST
S. aureus	Sensititre Broth microdilution	EUCAST

Appendix 6. Timeliness of AMR surveillance in Victoria

Surveillance System	Data provided to surveillance system	Public health follow up or reporting
AGAR	• Data are submitted to AGAR on a quarterly basis • Complete results, including typing, take up to 12 months to be reported back to contributing laboratories • Contributing laboratories submit specimens for typing and WGS on a quarterly basis	AGAR publishes annual reports; 2017 report was not published until early 2019
AURA	• The timeliness of AURA is affected by the timeliness of each contributing system.	None
CARAlert	• Once the isolate has been received by the confirming laboratory, additional testing is conducted within five working days (10). • Due to batching of some isolates, such as by the NNN and AMRL, some confirmatory testing results are not entered until two months after collection of the isolate (25). • MDU currently enters CARAlert data into the web portal once weekly/fortnightly. This may mean that isolates are not entered into the system for seven to 14 days after confirmatory testing has been completed	Bimonthly data updates and annual reports, fortnightly updates sent to DHHS and selected laboratories • Only five laboratories of 11 surveyed indicated that they currently receive regular CARAlert updates.
NNDSS	• Data submitted quarterly from DHHS to NNDSS	Latest *M. tuberculosis* report available online at the time of writing was for 2014 For *S. pneumoniae*, the latest available reports including AST data were dated 2011 and 2012

NNN	• Data submitted by laboratories quarterly to coordinating centre in NSW	2017 annual report was not available online until April 2019 for gonorrhoea As of April 2019, the 2017 *N. meningitidis* annual report could not be located online
VHPSS	• Most samples being entered within a fortnight of receipt at MDU • Delays in data entry can occur as some laboratories submit data in batches rather than continuously	No timely reporting or case follow-up
Victorian AMR unit	• MDU conducts AST twice weekly • AST takes from 8-48 hours to complete (depending on the rate of growth of the organism being tested) • Once testing is completed, a spreadsheet of AST results can be prepared on the same day • On identification of AMR as defined in (Appendix 2. Case definitions for AMR surveillance) MDU contacts the Principal Epidemiologist for AMR and WGS immediately	AST data received by the DHHS quarterly for *N. gonorrhoea* and fortnightly for *Salmonella* spp. and *Shigella* spp • On identification of critical AMR gonorrhoea isolates, a Partner Notification Officer or PHO conducts a risk assessment within 24 hours of notification
	VCRSU • Suspected CPE isolates are reported to VICNISS and/or DHHS/MDU within one business day. • Laboratories notify infection control staff and treating clinician immediately • CPE cases entered into PHESS within six hours of receipt by DHHS	• VICNISS usually obtains data on patient hospital movements within 24 hours of confirmation of a CPE case • As CPE infection control processes are now well-established, the healthcare facility has usually begun screening and contact tracing prior to being directed to do so
	TB	• Infectious TB notifications are entered into PHESS within six hours of receipt and referred to a public health nurse on the same day

	• All cases of TB must be notified to the DHHS within five days of diagnosis.	• TB notifications are checked by the TB epidemiologist daily and are couriered to the TB programme at Melbourne Health by 2pm on the same day • MDR and extremely drug resistant TB cases are entered into PHESS on the same day of notification. For infectious (pulmonary) TB, a meeting with the patient is arranged by Melbourne Health within three days of receiving notification

Appendix 7. Data collected by international systems for the surveillance of AMR within gonorrhoea

Gonococcal Isolate Surveillance Project, Centers for Disease Control and Prevention (49)

- Sex of patient's sexual partners within the past three months
- Travel history overseas in the past 60 days
- Sex work exposure in the past 12 months – defined as exchange of sex for drugs, money or exchange of drugs or money for sex
- Previous antibiotic use in the past 60 days for any reason, including systemic oral or injectable antibiotics
- Injectable drug use in the past 12 months
- Non-injectable drug use in the past 12 months (e.g. Ecstasy, cocaine)
- Primary treatment (including the strength) – trade name of drug is included as options
- Other additional treatment including name and dose
- Other additional treatment including treatment for chlamydia

European Gonococcal Antimicrobial Surveillance Programme (47)

- Previous gonorrhoea diagnosis
- Other STI diagnosed during current episode (all STIs)
- Probable country of infection – countries visited during incubation period of reported disease
- Mode of transmission: heterosexual, MSM, mother to child, other, unknown

Chapter IV: A Cross-sectional Study of the Genomic Epidemiology of Vancomycin-resistant Enterococci, Victoria, November 2018

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1. Preface

This project was initiated by Professor Benjamin Howden, Director of the Microbiological Diagnostic Unit Public Health Laboratory (MDU). The first snapshot study of vancomycin-resistant enterococci (VRE) in Victoria was conducted in November 2015. The prevalence of *van*A VRE was thought to have increased in Victoria since this time. In addition, there were concerns that *vanA* VRE may be spreading within and between healthcare facilities (HCF). The 2018 study was therefore planned to provide enhanced understanding of the current genomic epidemiology of VRE, enabling changes since 2015 to be examined. In addition, evidence for transmission within and between HCF would be assessed.

1.1 My role

My role included:

- Writing an ethics application, which was submitted to both the University of Melbourne and Australian National University Human Research Ethics Committees
- Contributing to the design of the isolate submission form, for participating laboratories to submit with isolates to be included in this study
- Liaising with staff from participating diagnostic laboratories in Victoria to arrange submission of isolates and the required data to MDU
- Extracting study data from the Laboratory Information Management System (LIMS)
- Cleaning data and transferring it into a Microsoft Access database to enable data management
- Liaising with MDU bioinformatics to conduct genomic analyses
- Combing the genomic analyses with epidemiological data by annotation of phylogenetic trees in R Studio
- Writing up findings for this chapter and presenting findings from this work at the Australian Epidemiological Association (AEA) Annual Scientific Meeting 2019 and the Communicable Disease Control Conference 2019

1.2 Lessons learnt

This project enabled me to learn an array of new skills including writing an ethics application, coordinating data collection, and cleaning data. I learned how genomic data can be used to inform future surveillance and public health response activities. I also learned how to annotate and interpret phylogenetic analyses in combination with epidemiological data.

1.3 Public health implications

This study found that there has not been an overall increase in the prevalence of *van*A vancomycin-resistant *Enterococcus faecium* (VREfm) in Victoria since 2015. The results of this study, combined with those of the 2015 study, provide baseline data on the prevalence of *van*A VREfm, in addition to the distribution of *E. faecium* sequence types found within Victoria. This study has also confirmed the value of the use of whole genome sequencing (WGS) data for surveillance of VRE. WGS enabled discrimination of VRE isolates beyond the multi-locus sequence type (MLST) level. Combining phylogenetic analyses with available epidemiological data indicated multiple likely episodes of transmission within and between HCF in Victoria.

In future, the combination of phylogenetic analyses with additional epidemiological data (such as patient admissions and ward movements) could be used to confirm putative transmission pathways for VREfm, or identify pathways not previously considered. This would inform appropriate measures to limit the spread of outbreaks. At the time of writing, the regulations of the *Victorian Public Health and Wellbeing Act 2008* were under revision. As of 2020, colonisation or infection with *van*A VRE will become a notifiable condition in Victoria. Using WGS and epidemiological data, a surveillance and response approach similar to that currently used for carbapenemase-producing Enterobacterales (discussed in Chapter III: AMR Surveillance in Victoria) could be adapted to *van*A VREfm in future.

1.4 Literature review

This Chapter satisfies the MAE requirement for conducting a literature review. Two databases were used for the literature search: the National Center for Biotechnology Information's PubMed and Google Scholar. The following combinations of keywords were searched: "vancomycin resistant enterococci" or "VRE", followed by "AND", combined with the terms "outbreak", "outbreak Australia", "MLST", "surveillance", "genomics", "whole genome sequencing", and "emergence". The literature review was an iterative process with references within articles sought out where relevant. In addition, I also assessed the grey literature relevant to this topic by conducting a google search in order to assess activities conducted by surveillance systems globally and locally. This identified surveillance activities conducted by the Australian Group on Antimicrobial Resistance, the European Antimicrobial Resistance Surveillance Network and the Centers for Disease Control and Prevention's National Healthcare Safety Network.

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- Kerrie Stevens for assistance with sample submission and coordinating WGS
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2. Abstract

Background: VREfm colonises patients and can cause serious infections, particularly within HCF. In Australia, *van*B VREfm predominates, however the prevalence of *van*A VREfm are thought to be increasing. Compared to *van*B VREfm, *van*A VREfm are more highly vancomycin resistant and display resistance to teicoplanin.

We conducted a cross-sectional study to investigate the genetic diversity and prevalence of VREfm within Victoria, Australia, in 2018, and compared findings to a similar 2015 study. We combined WGS and epidemiological data to assess for evidence of transmission within and between HCF.

Methods: During November 2018, Victorian diagnostic laboratories were requested to submit all VREfm cultures (diagnostic and screening samples) to MDU. WGS was performed and *in silico* MLSTs and *van* genotypes were identified. Descriptive analyses were conducted using Stata 15.1. Differences in proportions were assessed using a two-tailed Z test. Phylogenetic relatedness of isolates was analysed and compared with HCF data.

Results: In total, 311 isolates from 304 patients were identified. The incidence of VREfm infection in November 2018 was 0.15 per 100,000 population per month, similar to 2015 results (0.16 per 100,000 per month (p=0.60). 796 was the dominant MLST in both years (182 [59%] of 311 VREfm isolates, 2018, predominantly *vanB*). Among patients with VREfm, *van*A was detected in 64 (21%) of 311 isolates, compared to 55 (19%) of 293 isolates in 2015 (p=0.71).

MLST 1424 *van*A isolates were identified in Victoria in 2018 in 11 (28%) of 40 HCF. This MLST was not present in the 2015 study. Of the 33 MLST 1424 *van*A isolates, 32 (97%) fell within three closely-related genomic clusters, indicating spread within and between HCF.

Conclusions: The incidence of VREfm and the proportion of *van*A VREfm were similar in the 2015 and 2018 studies. The identification of MLST 1424 *van*A VREfm clusters indicates the need for additional patient screening and management to prevent further spread of VREfm.

3. Background

Enterococci are a normal part of the human gastrointestinal tract (1, 2). While enterococci have intrinsic or natural resistance to several antibiotics, they can also acquire new resistance genes and mutations which may confer resistance to additional antibiotics (1-4). Enterococci with genes conferring vancomycin resistance are known as vancomycin-resistant enterococci (VRE). There are nine known *van* genes among *Enterococcus* spp., *van*A, B, C, D, E, G, L, M and N (5). VRE includes the species *Enterococcus faecium* and *Enterococcus faecalis,* however the majority of VRE are *E. faecium* (3, 6, 7). Throughout this chapter, the term VRE is used to refer specifically to vancomycin-resistant *E. faecium* (VREfm).

Vancomycin was previously used to treat patients with enterococcal infections and penicillin-resistant *Staphylococcus aureus* (2, 8). Resistance to vancomycin was first identified in *E. faecium* in the 1980s, in Europe, and the USA (1, 9). VRE has now been identified throughout Europe, the United Kingdom, Japan, and Australia (9). In Australia, outbreaks of VRE have been reported in Western Australia, South Australia, the Australian Capital Territory, and Victoria (10-13).

3.1 Public health significance

Most people who acquire VREfm become carriers (a state known as colonisation), however those with immunosuppression or comorbid conditions can develop significant infections (14). Enterococcal infections can manifest as urinary tract infections, intra-abdominal infections, blood-stream infections, endocarditis and postoperative infections (3, 15).

Compared to infections caused by vancomycin susceptible bacteria, VREfm infections are more difficult to treat and are associated with higher levels of morbidity and mortality (3, 16). In addition, the management of patients infected or colonised by VREfm within HCF is associated with increased healthcare costs (17).

The World Health Organization has listed VREfm as high priority for the research and development of new antibiotics (18). VREfm is resistant to commonly used antimicrobials (e.g. ampicillin), and treatment therefore relies on last-line antibiotics such as daptomycin (6, 7, 14, 19). There have only been two new antimicrobials developed for the treatment of VRE since its emergence; quinupristin/dalfopristin and linezolid (7). Quinupristin/dalfopristin requires placement of a central line, a type of catheter which can cause complications, and carries a number of side effects, while the effectiveness of daptomycin is considered to be unclear (7).

Concerningly, resistance to newer, last-line antibiotics such as daptomycin is emerging among VRE (20).

Enterococci can colonise the gastrointestinal tract, urinary tract, medically implanted devices such as catheters, and chronic wounds (1, 7). Risk factors for colonisation include severe medical conditions resulting in long-term hospitalisation, haematologic malignancies, organ transplantation and long-term haemodialysis, the presence of invasive medical devices (e.g. indwelling urinary catheters), and previous treatment with broad-spectrum antimicrobials (14, 21). Patients who are colonised by VRE do not display any symptoms, but shed the organism in faeces, and carry it on their skin (3, 22)

Among colonised patients, the development of a VRE infection is associated with admission to wards including ICU, acute, haematology or oncology, and undergoing dialysis or organ transplantation (3, 14). The proportion of colonised patients who go on to develop infections is variable, ranging from 0-45% (23, 24).

Patients can acquire VRE from other colonised or infected patients, or commensal enterococci within the intestine can acquire vancomycin resistance when exposed to broad-spectrum antibiotics or other bacteria carrying vancomycin-resistance genes (14). Furthermore, VRE can transfer resistance genes to both related and unrelated strains of bacteria, including *Staphylococcus aureus* (3, 14).

VRE are difficult for hospitals to control as the organism can spread via direct contact with infected or colonised patients or on the hands of healthcare staff, as well as through indirect contact with contaminated environmental surfaces such as medical equipment within a HCF (3, 14). Once a healthcare environment is contaminated with VRE, the organism can remain viable for months (1, 14). In addition, outbreaks of VRE within hospitals may be due to a subpopulation of *E. faecium* adapted to the healthcare environment (7). Attempts to control the spread of VRE therefore focus on disinfection, screening of patients to identify those colonised by VRE, and isolation of colonised and infected patients (9).

3.2 Vancomycin-resistant mechanisms and genomics

All bacteria have cell walls made up of compounds known as peptidoglycans. Glycopeptide antimicrobials, including vancomycin and teicoplanin, work by binding the outer surface of the cell membrane, which prevent the production of peptidoglycans and the formation of a cell wall (7). Resistance to glycopeptides occurs through the production of an alternative peptidoglycan precursor by bacteria (7).

Of the *van* genes encoding glycopeptide resistance, *van*A and *van*B are the two main types conferring vancomycin resistance within VREfm (7, 25, 26). Organisms expressing the *van*B gene are resistant only to vancomycin, whereas those expressing the *vanA* gene have high-level resistance to vancomycin in addition teicoplanin resistance (3, 7, 26, 27).

3.3 MLST and WGS

Multi-locus sequence typing (MLST) generally provides sufficient discrimination of VRE within HCF in order to assist with the identification of outbreaks. MLST assigns a four-digit code (e.g. 1421) on the basis of the alleles present in seven housekeeping genes, with each allele assigned an integer (6, 28). Historically, MLST was conducted by performing multiple polymerase chain reaction tests targeting the loci of housekeeping genes (28). While used to infer outbreaks of VREfm, MLST lacks the level of detail required to identify all possible transmission pathways (5).

The genome of *E. faecium* has high levels of recombination, whereby DNA is exchanged between organisms (15, 29). Recombination can affect the loci included in the MLST scheme, meaning that an isolate's MLST may be reclassified once recombination is accounted for, indicating that the use of MLST alone is inadequate for examining the genomic epidemiology of VREfm (15, 30). WGS approaches enable isolates to be characterised to a higher level than MLST (31). Furthermore, the use of WGS data combined with epidemiological data can enable the identification of transmission pathways which can be used to inform appropriate public health actions (5).

3.4 Australian context

In Europe and the United States, *van*A VREfm predominates (26). In contrast, in Australia, *van*B VREfm predominates however the prevalence of *van*A VREfm is thought to be increasing (25, 26, 32). The prospect of increasing prevalence of *van*A VREfm is concerning as these isolates are associated with higher minimum inhibitory concentrations (MICs) for vancomycin in addition to teicoplanin resistance (Table 1) (7, 33). Higher MICs mean that infections require higher doses of antibiotics (7, 32, 34).

Table 1. Comparison of van genotype characteristics (7, 33, 35)

Genotype	Vancomycin MIC	Teicoplanin MIC
*van*A	High level resistance >256mg/L	Resistant >64mg/L)
*van*B	Resistance 16-32 mg/L	Susceptible ≤2 mg/L
European Committee of Antimicrobial Susceptibility Testing breakpoints	Resistance >4 mg/L Susceptibility ≤4 mg/L	Resistance >4 mg/L Susceptible ≤2 mg/L

The Australian Group on Antimicrobial Resistance (AGAR) conduct sentinel surveillance of enterococcal infections. In AGAR's *Australian Enterococcal Sepsis Outcome Program (AESOP) 2017* report, there is a documented increase in the proportion of VREfm isolates with teicoplanin resistance in Australia, and an increase in the proportion of VREfm resistant to both vancomycin and teicoplanin in Victoria (19). Annual reports from AGAR have documented an increase of the proportion of VREfm with *van*A from 20.1% in 2015 to 24.9% in 2017 in Australia, and from 12.5% in 2015 to 14.9% in 2017 in Victoria (36).

VRE was first identified in Victoria in 1994 and is now widespread within healthcare institutions (37). While AGAR sentinel surveillance enables monitoring of long-term trends for VRE, data are not representative for Victoria as only five large HCF contribute to the AGAR scheme. In 2018, 130 *E. faecium* isolates from blood-stream infections (BSI) were submitted to AGAR, with 61.5% reported as vancomycin non-susceptible (intermediate susceptibility and resistance combined). Conversely, in the same time period, 195 *E. faecium* isolates from BSI were submitted to the Victorian Hospital Pathogen Surveillance Scheme (VHPSS) (38). Of 189 isolates submitted to the VHPSS with vancomycin susceptibility data, 59.8% were non-susceptible to vancomycin (38). Both AGAR and VHPSS data are limited to blood culture isolates, therefore a comprehensive understanding of VREfm epidemiology is not able to be obtained from these systems.

3.5 2015 VRE snapshot study

In November 2015, MDU ran the first laboratory based cross-sectional study of VRE in Victoria. This study performed WGS on all VREfm specimens from 96% of Victorian laboratories over a 30-day period. This enabled an assessment of the genetic factors associated with vancomycin resistance and the phylogenetic relationships between isolates (37). The 2015 snapshot study

identified *van*B VREfm in 42 of 47 HCF (89.4%), while *van*A VREfm were only found in 11 of 47 HCF (23.4%) (37).

The 2018 snapshot study followed the methodology of the 2015 study, enabling changes between the two time periods to be identified. As VREfm from all specimens were included in both studies, this assisted with further characterisation of the burden of disease associated with VREfm in Victoria. The inclusion of both private and hospital-based laboratories in both studies enabled an assessment of community-acquired VREfm in addition to health-care acquired VREfm. Vancomycin sensitive *E. faecium* (VSEfm) bacteraemia isolates were included in both the 2015 and 2018 studies as they have been suggested to be important in the emergence of VREfm and should be included in efforts to control VRE spread (15).

3.6 Objectives

With this study we aimed to:

- Investigate changes in the prevalence and genomic diversity of VREfm that have occurred between November 2015 and November 2018 in Victoria
- Determine if the prevalence of *van*A VREfm had increased in Victoria between the 2015 and the 2018 studies
- Describe the epidemiology of VREfm in Victoria in November 2018
- Evaluate the presence of transmission networks within and between HCF in Victoria in November 2018

4. Methods

All Victorian diagnostic laboratories were asked to submit to MDU all VREfm, from any diagnostic or screening sample, and all vancomycin-susceptible *E. faecium* (VSEfm) from blood cultures, from samples collected from 01 to 30 November 2018. Participation by laboratories was voluntary.

Isolates were submitted with either a hardcopy or electronic submission form. The submission form included patient sex, unit record number, the site of specimen collection, HCF where the specimen was collected, date of collection, and whether the specimen was collected for clinical or screening purposes.

4.1 Laboratory methods

Diagnostic laboratories routinely screen isolates of *E. faecium* for phenotypic susceptibility to vancomycin. In addition, a polymerase chain reaction is conducted to ascertain the presence or absence of *van* genes. Based on the results of initial screening tests, isolates that met the criteria for the study were submitted to MDU for WGS. Submitted specimens were stored at 4°C until laboratory testing was completed. Single colony isolates used for WGS were frozen in glycerol storage broth at -70°C. Genomic DNA from pure isolates was extracted using the JANUS automated workstation with the Chemagic viral DNA/RNA kit (PerkinElmer, Waltham, USA). Unique dual indexed libraries were prepared using the Nextera XT DNA sample preparation kit (Illumina, San Diego, USA). Libraries were sequenced on the Illumina NextSeq 500 with 150-cycle paired-end chemistry.

4.2 Bioinformatic analysis

Illumina sequence reads were analysed using the public health bioinformatics pipeline, *Nullarbor* (39). Outputs from the *Nullarbor* pipeline include genome assembly, *in silico* MLST, detection of *van* genes, and phylogenetic analyses. Phylogenetic trees were constructed from core genome single nucleotide polymorphisms (SNPs) identified by mapping reads to the *E. faecium* reference genome, Aus0085 (accession no. NC_021994).

For sequence types where more than ten isolates were identified (MLST 17, 78, 80, 203, 796, 1421 and 1424), a phylogenetic tree was constructed for isolates within that MLST, using the following reference genomes: MLST 17: AUSMDU0000004 (accession no. CP003351), MLST 78: AUSMDU00004055 (accession no. CP027506), MLST 80: AUSMDU00004142 (accession no. CP027501), MLST 796: AUSMDU00004028 (accession no. CP027512), MLST 203:

AUSMDU00000085 (accession no. CP006620), MLST 1421: AUSMDU00004167 (accession no. CP027497) and, MLST 1424: AUSMDU00011555 (no accession no. available).

4.3 Epidemiological analysis

All identifying patient information was removed and patient identification numbers were assigned in the order of isolate submission. HCF letter codes matched those used in the 2015 study, with additional letter codes assigned by order of submission. Isolates with no known HCF origin or those submitted by general practitioners were classified as 'community' isolates.

Without detailed medical information, the clinical significance of urine samples is often unclear as VREfm can colonise the urinary tract (40). Specimens from which isolates were derived were therefore classified as either clinical, colonisation, or urine. BSIs were examined as a subset of clinical infections, as they are a major cause of mortality (41).

Descriptive analysis was performed using STATA 15.1 (StataCorp LLC, Texas, USA). Incidence rates were calculated using the mid-year Estimated Resident Population for Victoria in relevant years (42). Differences in incidence rates and proportions were assessed using a two-tailed Z test. *P*-values less than 0.05 were considered to be significant.

Phylogenetic trees were annotated by MLST, HCF origin and *van* genotype using ggtree (43) in R Studio™ (RStudio Team, Boston, USA). Only MLSTs with more than ten isolates identified were highlighted, those with fewer than ten isolates were classified as "other" for visualisation.

SNPs are mutations of a single base pair within a genome (29). Pairwise SNP differences indicate the number of nucleotide changes between two isolates, with fewer SNPs indicating isolates more closely related to each other. While the assessment of pairwise SNP differences does not take into account multiple mutations at a nucleotide site, or differences in the rates of transitions or transversions, assessing potentially related isolates using SNP cut-offs using WGS data is commonly performed as part of surveillance and outbreak detection for a variety of communicable organisms (29). In this study isolates falling within clusters as defined by number of SNPs were annotated on each MLST level phylogenetic tree. This aspect of VREfm was not examined in the 2015 study, therefore SNP cut-off levels were defined with reference to previous outbreak investigations and studies of VREfm (13, 44-46). Clusters were annotated at two cut-off levels to indicate the degree of relatedness of isolates: 5 SNPs and 25 SNPs.

To examine the pairwise distribution of SNP differences for isolates within and between HCF, violin plots, which are similar to box plots with the addition of probability density, were created using R Studio™. Where two isolates originated from the same HCF, they were categorised as "Within HCF." Conversely, where two isolates originated from different HCFs, they were categorised as "Between HCF." Isolates classified as having a community origin were excluded from this part of the analysis. Isolates were separated into *van*A and *van*B genotypes for comparison. In addition, as the 2015 study found that MLST 796 *van*B displayed limited genetic diversity, *van*B was further separated into *vanB* VREfm excluding MLST 796 isolates, and *vanB* VREfm MLST 796 only. Differences in the pairwise SNP differences between and within HCF were assessed using the Mann Whitney Rank Sum test, while differences in the median pairwise SNP difference were assessed using a nonparametric *k*-sample test on the equality of medians.

5. Results

The majority of diagnostic laboratories (96%, 26/27) in Victoria participated in the study. While some laboratories used the study submission form, other laboratories sent isolates with routine laboratory reports. Upon exclusion of isolates (n=104) not meeting our study criteria, 316 isolates remained for analysis (Figure 1). Of these, 301 isolates were collected from 294 patients from 41 HCF. An additional fifteen patients had no known link to any HCF at the time their sample was collected (and are subsequently referred to as 'community' patients).

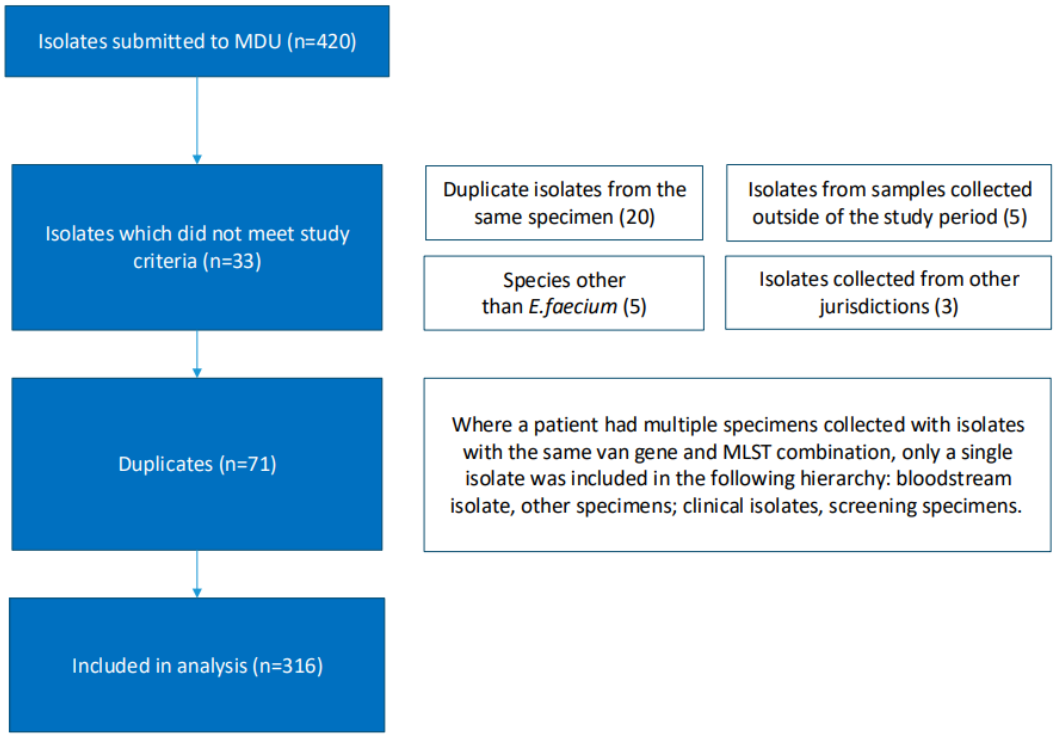

Figure 1. *E. faecium* isolates excluded from the 2018 VRE snapshot study

5.1 Has the prevalence of *van*A VREfm increased in Victoria between 2015 and 2018?

Of the 316 snapshot isolates, 311 (98.4%) were VREfm and five (1.6%) were VSEfm. Of 311 VREfm isolates, 20.6% (n=64) were *vanA* and 78.5% (n=244) were *van*B. Three isolates carried both the *vanA* and *vanB* mechanisms (*van*A+B). The was no significant difference in the proportions of *van*A and *van*B VREfm isolates in 2018 compared to that found in 2015 (81% *van*B and 19% *van*A in 2015, p=0.71).

5.2 Characterising the burden of disease of VREfm in Victoria

Patient specimens from which VREfm isolates were derived (n=311) are summarised in Table 2. This included:

- 45 VREfm from clinical infections in 45 patients
- 73 VREfm from 72 patient urine specimens
- 192 VREfm obtained from screening specimens in 187 patients
- One specimen collected for unknown purposes (treating clinician did not specify the reason for collection)

Among VREfm isolates classified as clinical infections, the most common specimen source was blood cultures (40%, n=20). Of specimens classified as colonisation, 83% were isolated from rectal swabs (n=158).

The median age for patients infected or colonised by VREfm was 70 years (range 0 to 97 years), 42% of patients (n=304) were female (n=127) and 58% were male (n=177). Of five patients with VSEfm bacteraemia, the median age was 72 years (range 60–94 years), three patients were males and two were females.

The incidence of VREfm clinical infection (including urine isolates) was 0.15 per 100,000 population per month. There was no statistically significant difference between the incidence rate of VRE infection in 2015 and 2018 (2015 rate of 0.16 per 100,000 per month, p=0.60). The incidence of *van*A VREfm infection or colonisation was 1.0 per 100,000 population per month, similar to 2015 (0.92 per 100,000 population per month) (p =0.72).

Table 2. Number of *E. faecium* isolates collected from specimen types by specimens classified as clinical, screening and urine.

Specimen classification					
Specimen type	**Clinical**	**Screening**	**Unknown**	**Urine**	**Total**
Abdomen tissue	1	0	0	0	1
Abdominal cavity fluid	1	0	0	0	1
Abdominal cavity swab	1	0	0	0	1
Abscess	1	0	0	0	1
Arm wound swab	1	0	0	0	1
Blood (whole)					
VREfm	20	0	0	0	20
VSEfm	5	0	0	0	5
Bone	1	0	0	0	1
Drain wound fluid	3	0	0	0	3
Faeces	0	7	0	0	7
Foot/ankle wound swab	1	0	0	0	1
Groin swab	0	3	0	0	3
Groin tissue	1	0	0	0	1
Hip swab	1	0	0	0	1
Hip tissue	1	0	0	0	1
Kidney (percutaneous nephrostomy)	1	0	0	0	1
Leg swab	1	0	0	0	1
Pancreas tissue	1	0	0	0	1
Perianal swab	0	1	0	0	1
Peritoneal cavity fluid	2	0	0	0	2
Pleural cavity fluid	2	0	0	0	2
Rectal swab	0	158	0	0	158
Swab (unspecified site)	1	22	1	0	24
Thigh swab	1	0	0	0	1
Tissue	2	0	0	0	2
Trachea aspirate	1	0	0	0	1
Urine	0	1	0	73	74
Total	**50**	**192**	**1**	**73**	**316**

Figure 2 summarises the specimen source and resistance genotype of all isolates. Of the 45 isolates causing clinical infections, 20% were due to *van*A VREfm (n=9) and 78% were due to *van*B VREfm (n=35). One patient (2%) had a clinical infection due to VREfm carrying both *van*A and B. There were no significant differences (p=0.63) in the proportion of *van* genotypes causing colonisation (18% *van*A, 35/192; 81% *van*B, 155/192; 1.0% *van*A+B, 2/192) and isolates from urine samples (*van*A, 27%, 20/73; 73% *van*B, 53/73).

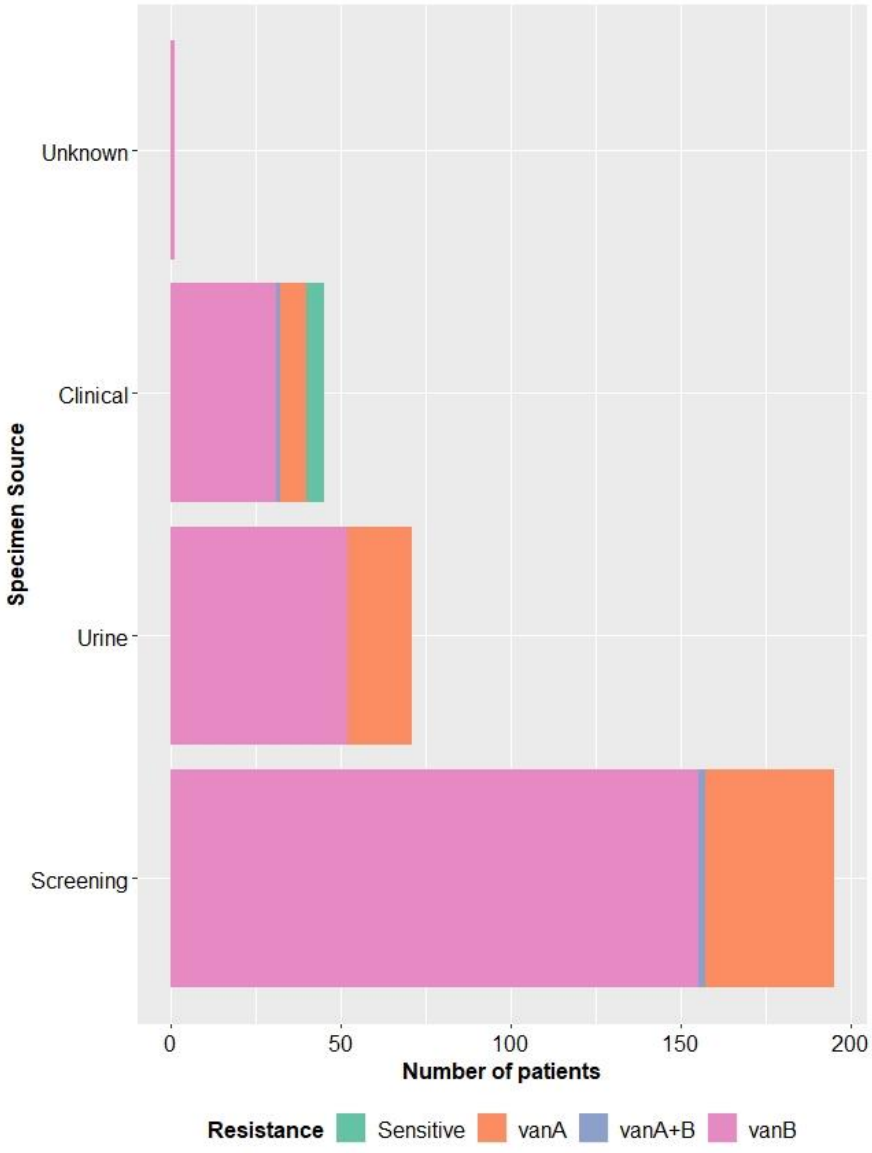

Figure 2. Number of *E. faecium* isolates by *van* genotype and specimen classification

5.2.1 *E. faecium* bacteraemia

E. faecium bacteraemia was identified in 25 patients during the study period. Of these patients, 20 had VREfm bacteraemia: 35% (n=7) had *van*A VREfm and 65% (n=13) had *van*B VREfm. The incidence of *E. faecium* bacteraemia in Victoria for the snapshot period was 0.38

per 100,000 per month, similar to the incidence found in 2015 (0.50 per 100,000 population per month, *p*=0.90). Of VREfm bacteraemia isolates, 44% (n=11) were *van*B MLST 796.

5.2.2 VREfm in Victorian HCF

VREfm were distributed across 40 HCF (Figure 3).

- Of all VREfm isolates, 32% were identified from HCF B (n=98/311)
- *van*A VREfm isolates originated from 13 of 40 HCF, and from four community patients
- Of 64 *van*A VREfm isolates, 31% (n=20) were identified by HCF B
- There were no statistically significant increases in the proportion of *van*A VREfm isolates originating from any individual HCF between 2015 and 2018 (data not shown)
- Clinical isolates were found in 18 HCF and two community patients.
    - Of these, bacteraemia isolates were found in 12 HCF and two community patients
- Urine isolates were found in 31 HCF and 12 community patients
- Screening isolates originated from 15 HCF and one community patient
- Of 192 isolates originating from screening samples, HCF B identified 47% of isolates (n=91)

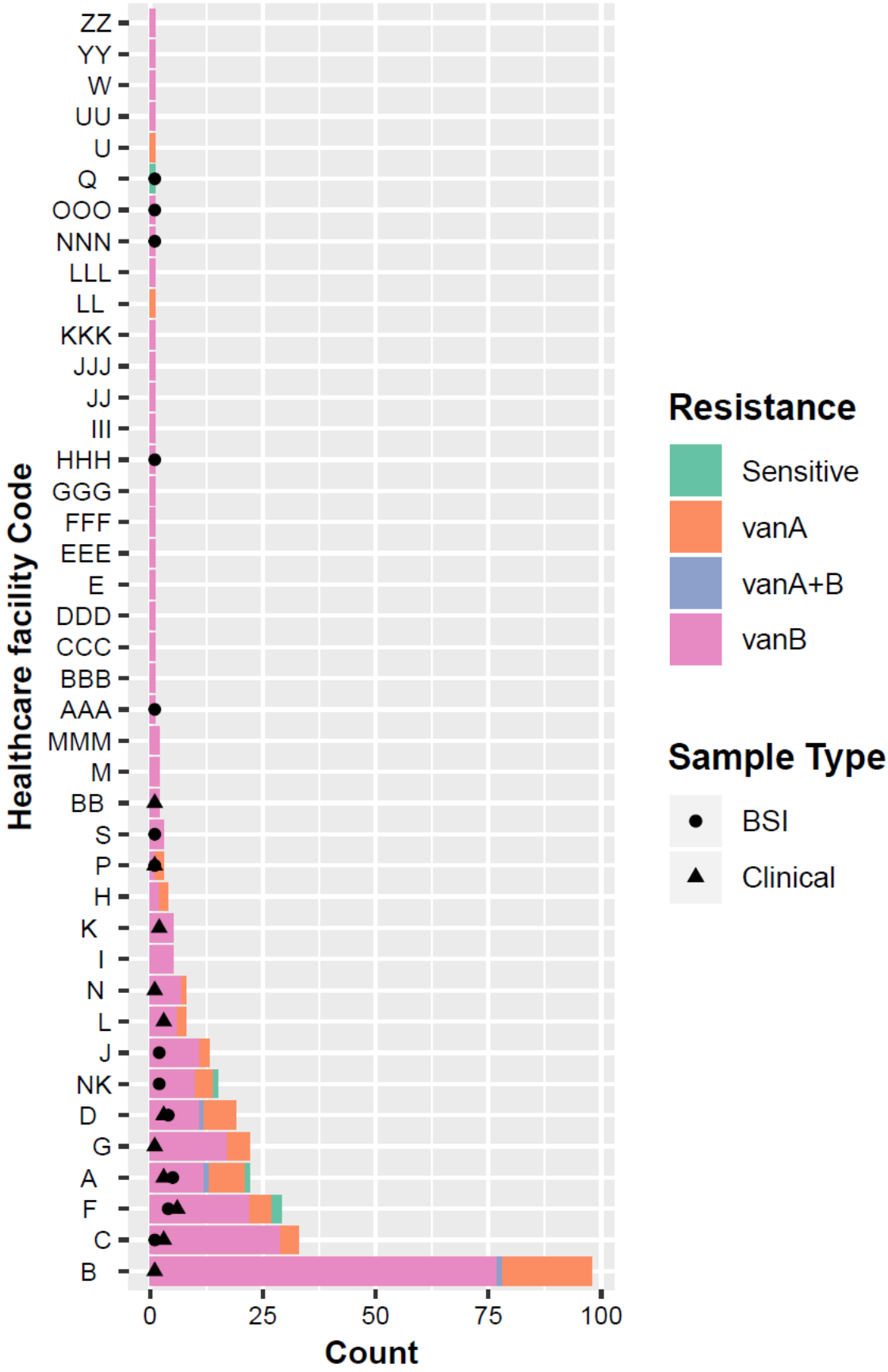

Figure 3. Number of *E. faecium* isolates by healthcare facility (HCF) and *van* genotype. Clinical isolates (including urine isolates) and bloodstream isolates (BSI) are shown. Letters indicate HCF code, with NK indicating isolates with no known HCF origin.

5.3 Changes in the genomic diversity of VREfm in Victoria between November 2015 and November 2018

One way to examine changes in genomic diversity is to assess MLSTs among isolates.

Overall, there were 16 MLSTs observed for VREfm (Table 3).

- Among all *van*A VREfm, there were nine MLSTs.
    - Of 64 vanA VREfm isolates, 51.6% were MLST 1424 (n=33) and 23.4% were MLST 1421 (n=15)
- Among *van*B VREfm, there were 13 MLSTs, in addition to a non-typeable group of three isolates (where alleles have not been able to be accurately identified, and therefore MLST has not been able to be assigned).
    - Of 244 vanB VREfm isolates, 74.2% were MLST 796 (n=181)

Among clinical samples, ML ST 1421 and 203 were most prevalent among *van*A isolates, while among screening specimens with *van*A, MLST 1421 and 1424 were most prevalent. Among *van*A isolates, MLST 1424 was most prevalent among urine samples (11/20, 55.0%). Among *van*B isolates, MLST 796 comprised the majority of clinical (24/35, 68.6%), screening (112/155, 72.3%) and urine (44/53, 83.0%) specimens.

Among *van*A+B, there were three isolates of three MLSTs: 252,796 and 992. Among VSEfm, there were four MLSTs: 17, 203, 71 and 928.

Table 3. MLST by van gene and specimen category[12]

	Specimen category					
MLST	**Clinical (except bacteraemia)**	**Bacteraemia**	**Screening**	**Urine**	**Unknown**	**Total**
***van*A**						
1424	0	3 (42.9%)	19 (54.3%)	11 (55.0%)	0	33 (51.6%)
1421	0	1 (14.3%)	8 (22.9%)	6 (30.0%)	0	15 (23.4%)
203	1	1 (14.3%)	2 (5.7%)	1 (5.0%)	0	5 (7.8%)
80	0	1 (14.3%)	3 (8.6%)	1 (5.0%)	0	5 (7.8%)
17	1	0	1 (2.9%)	0	0	2 (3.1%)
1489	0	0	0	1 (5.0%)	0	1 (1.6%)
262	0	1 (14.3%)	0	0	0	1 (1.6%)
761	0	0	1 (2.9%)	0	0	1 (1.6%)
Novel 1	0	0	1 (2.9%)	0	0	1 (1.6%)
Total	**2**	**7**	**35**	**20**	**0**	**64**
***van*B**						
796	13 (59.1%)	11 (84.6%)	112 (72.3%)	44 (83.0%)	1	181 (74.2%)
78	2 (9.1%)	0	11 (7.1%)	3 (5.7%)	0	16 (6.6%)
80	0	1 (7.7%)	9 (5.8%)	2 (3.8%)	0	12 (4.9%)
17	3 (13.6%)	0	6 (3.9%)	0	0	9 (3.7%)
203	0	0	4 (2.6%)	1 (1.9%)	0	5 (2.0%)
Non-typeable	1 (4.5%)	0	2 (1.3%)	0	0	3 (1.2%)
1421	1 (4.5%)	0	2 (1.3%)	0	0	3 (1.2%)
555	0	0	0	3 (5.7%)	0	3 (1.2%)
1283	0	0	2 (1.3%)	0	0	2 (0.8%)

[1] Novel MLSTs indicate MLSTs not yet assigned

[2] Non-typeable MLST indicates isolates were not all alleles were able to be identified in order to assign an MLST

252	1 (4.5%)	0	1 (0.6%)	0	0	2 (0.8%)
Novel 3	0	0	4 (2.6%)	0	0	4 (0.8%)
992	0	0	1 (0.6%)	0	0	1 (0.4%)
Novel 1	1 (4.5%)	0	0	0	0	1 (0.4%)
Novel 2	0	1 (7.7%)	1 (0.6%)	0	0	2 (0.4%)
Total	22	13	155	53	1	244
***van*A+B**						
252	1	0	0	0	0	1
796	0	0	1	0	0	1
992	0	0	1	0	0	1
Total	1	0	2	0	0	3
VSEfm						
17	0	2	0	0	0	2
71	0	1	0	0	0	1
203	0	1	0	0	0	1
928	0	1	0	0	0	1
Total	0	5	0	0	0	5

Seven patients had more than one isolate with two different MLSTs or two *van* gene types (Appendix 1); five of these patients were from the same HCF (HCF B). Of note, HCF B was conducting a point prevalence survey for VREfm during the study period, which involved screening all patients at risk of VREfm.

Of the patients with multiple isolates, most samples (11/14, 78.6%) were obtained from screening tests, and most (10/14, 71.4%) were obtained either on the same day or one day apart. Four of these patients were simultaneously colonised or infected with a *van*A and a *van*B isolate.

The distribution of MLSTs among *E. faecium* has changed significantly between 2015 and 2018 (p<0.001). Notably, MLST 1424 *van*A VREfm was not present in the 2015 study (Figure 4). Among *van* B VREfm, the proportion of MLST 78 (p<0.001) and 80 (p=0.03) increased, and MLST 203 (p=0.003) decreased (Figure 4). There was no difference in the proportion of *van* B VREfm MLST 796 (p=0.24) or MLST 17 (p=0.35). MLST 1421 was not present in the 2015 study among *van*B VREfm.

Among *van* A VREfm, the proportion of MLST 80 and 203 decreased (p<0.001). There was no change in the proportion of MLST 1421 (p=0.12). MLST 1424 and 17 were not present in the 2015 study among *van*A VREfm.

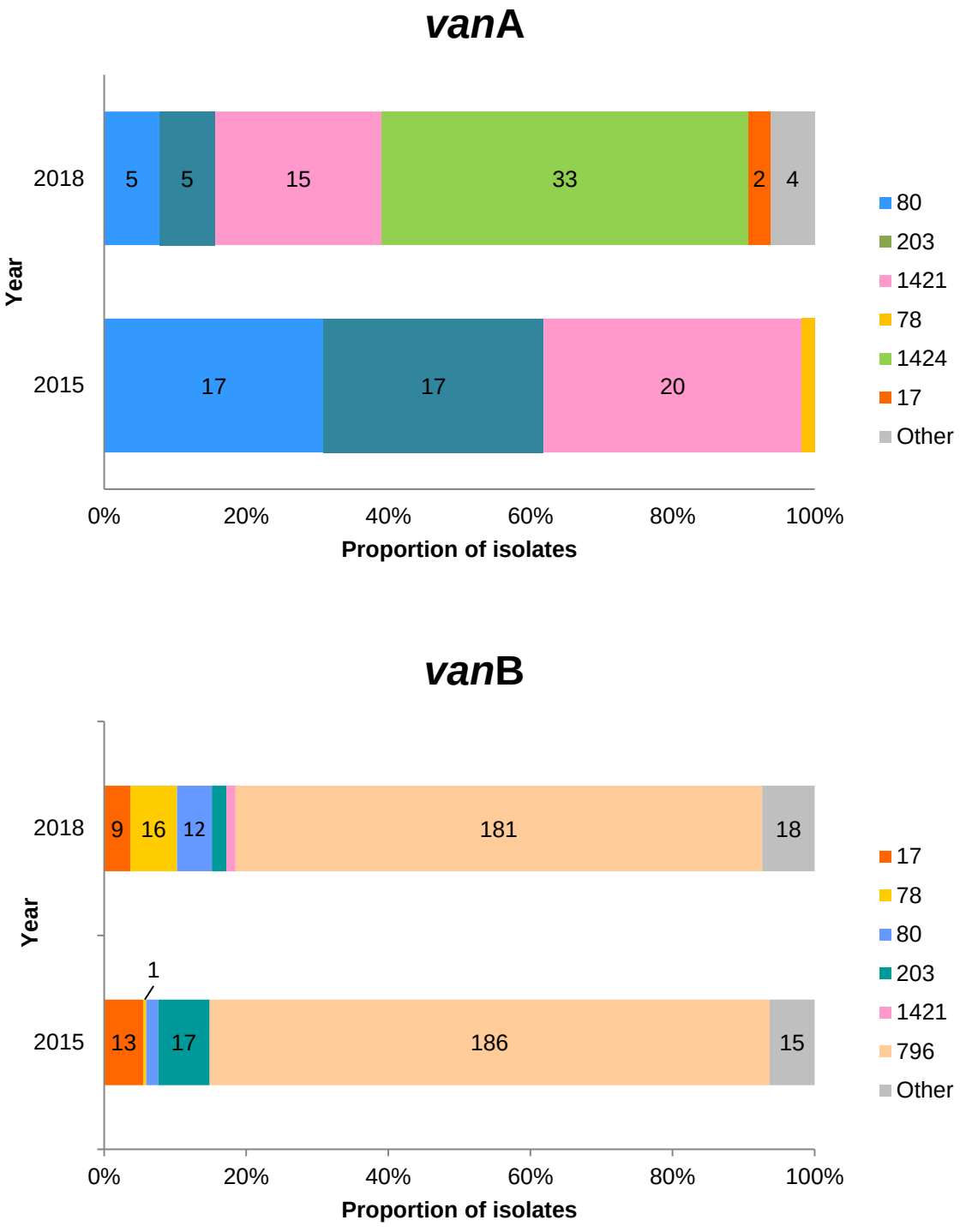

Figure 4. Proportion of MLST among *vanA* and *vanB* VREfm, 2015 and 2018[3]

[3] Other MLST are those were less than 10 isolates were identified during November 2018

MLST 1424 comprised the majority of vanA VREfm. MLST 1424 originated from 11 HCF and one community patient (Figure 5).

- Of 34 MLST 1424 isolates, 38% (n=13) were isolated from HCF B
- All MLST 1424 isolates were *van*A VREfm
- Of 34 MLST 1424 isolates, 56% (n=19) originated from screening samples, and 32% (n=11) originated from urine samples

MLST 796 comprised the majority of vanB VREfm. MLST 796 originated from 33 HCF. In addition, there were nine community patients.

- Of 182 MLST 796 isolates, 62% (n=113) originated from screening samples
- Except for a single *van*A+B isolate, all MLST 796 were *van*B
- Of 182 MLST 796 isolates, 29% (n=52) originated from HCF B

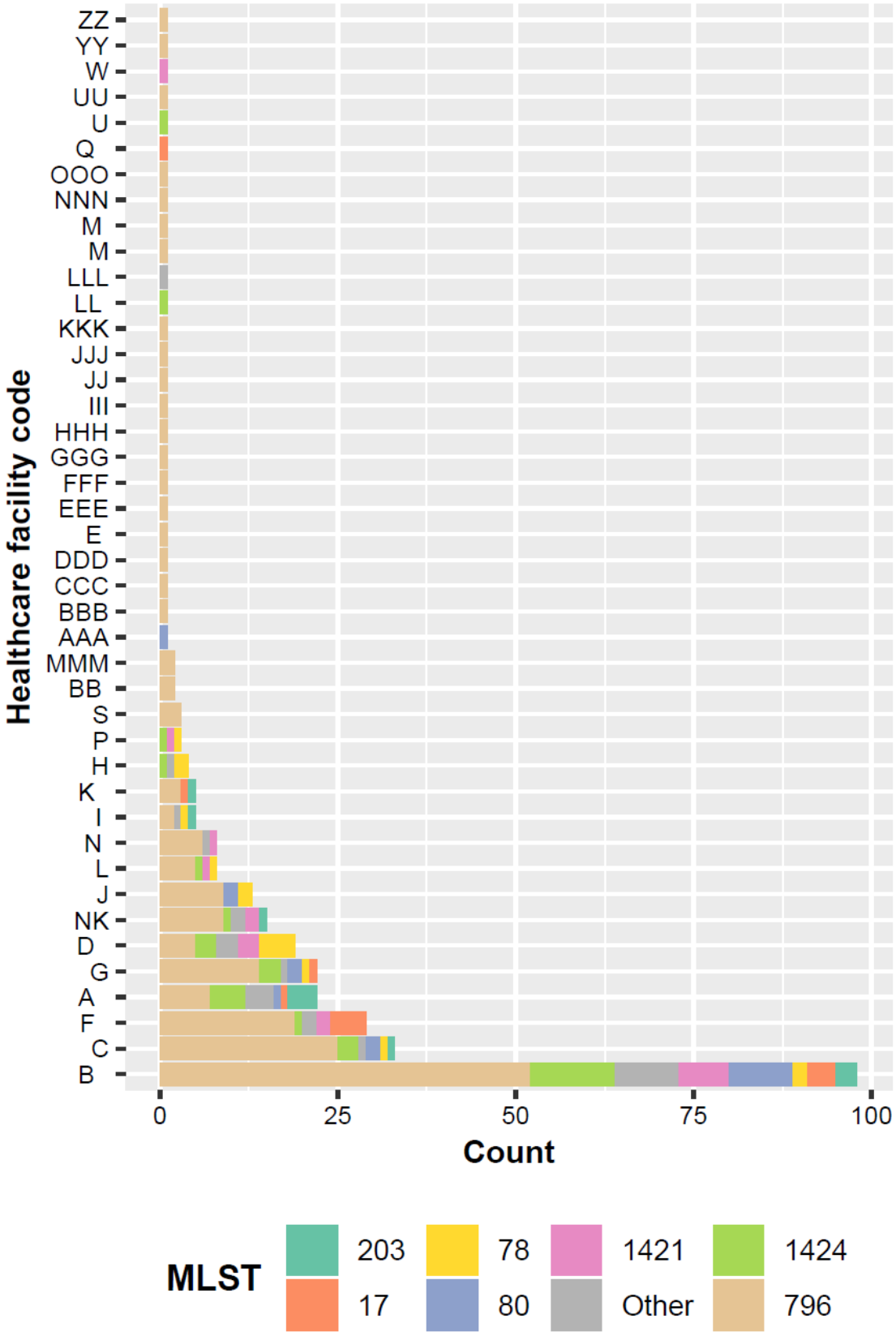

Figure 5. Number of *E. faecium* isolates by MLST and Healthcare facility (HCF). MLSTs with less than ten isolates identified were classified as "other."

5.4 Evaluating the presence of transmission occurring within and between HCF in Victoria

Figure 6. displays the genomic relationships of *E. faecium* isolates together with the MLST, HCF, and *van* genotype. Most isolates were part of a large monophyletic group with several distinct branches outside of this group. Isolates clustered mostly by MLST. Notably, there were multiple large clusters of MLST 796, spread across the majority of HCF. There were three clusters of MLST 1424, spread across several HCF. Interestingly, HCF B has identified at least one isolate of each of the most prevalent sequence types (MLSTs with more than ten isolates in the study).

Phylogenetic analysis identified two divergent isolates originating from two different HCF. These isolates had a distance of more than 10,000 SNPs from other isolates in the study. One isolate was *van*A, with a novel MLST, and the other was VSEfm, MLST 928. For useful visualisation, phylogenetic trees are presented excluding these isolates.

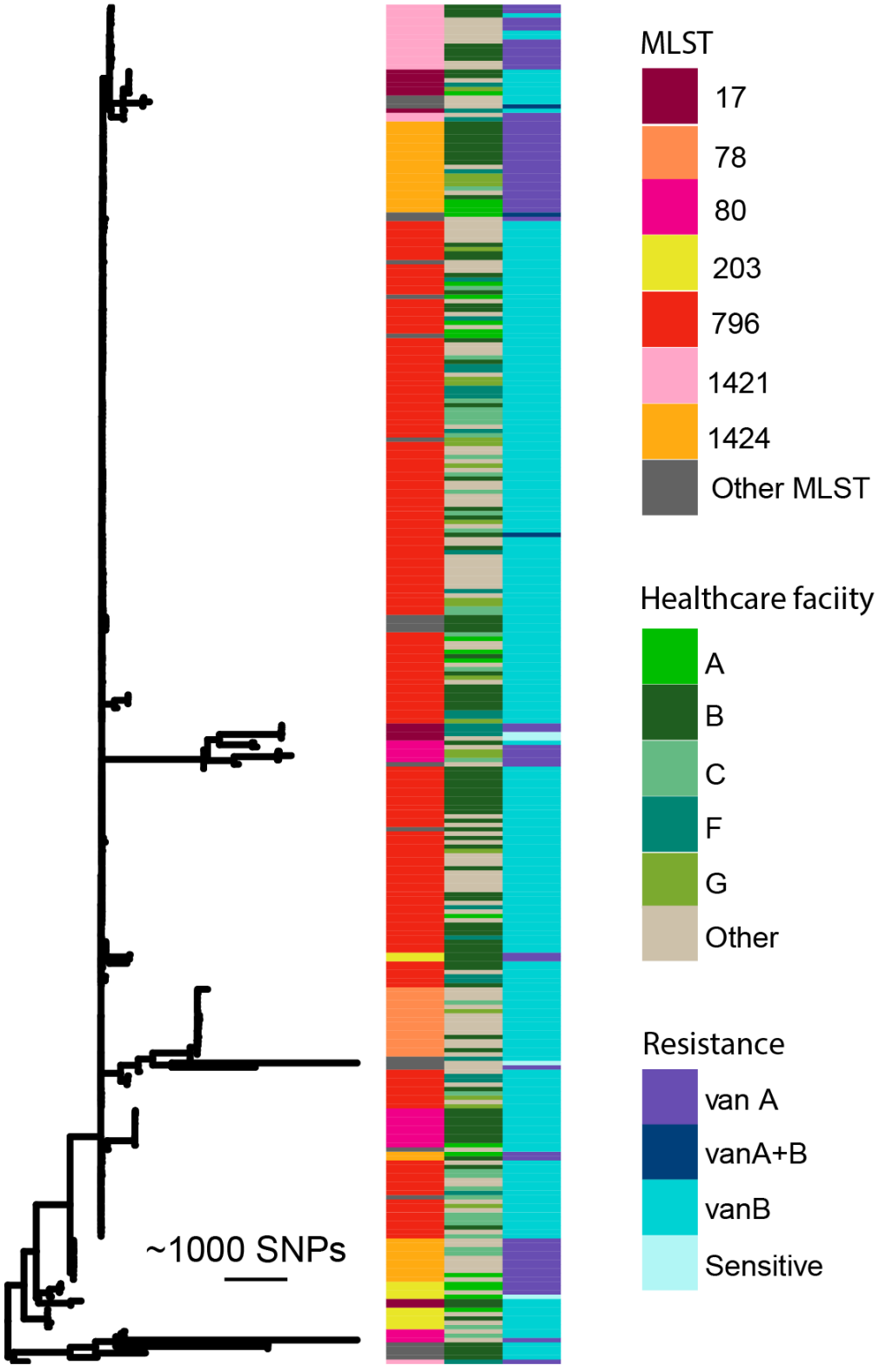

Figure 6. Phylogenetic tree displaying 314 *E faecium* isolates. Two highly divergent isolates have been excluded to enable data visualisation. The three columns adjacent to the phylogenetic tree display MLST, healthcare facility (HCF) and *van* gene. Only MLSTs with more than ten isolates are displayed. Only HCF with more than 20 isolates are shown.

One method to evaluate the presence of transmission within or between HCF is to use SNP cut-off levels to define clusters. Where clusters include isolates which originate from different HCFs, this could indicate transmission occurring between HCFs in Victoria. While several clusters were identified within each MLST examined, only MLST 1424 (present in the 2018, but not the 2015 study) and 796 (comprising the majority of *van*B VREfm) are included here to demonstrate the identification of putative transmission events in Victoria. Other MLSTs are included in Appendix 2.

Among MLST 1424, 32 of 33 isolates fell within three distinct clusters at the 25 SNP level (Figure 7).

- Within these clusters, there were multiple clusters at the 5 SNP level
- Cluster one included eight HCF
- Cluster two included six HCF, and one community patient
- Cluster three included 11 isolates isolated from HCF B

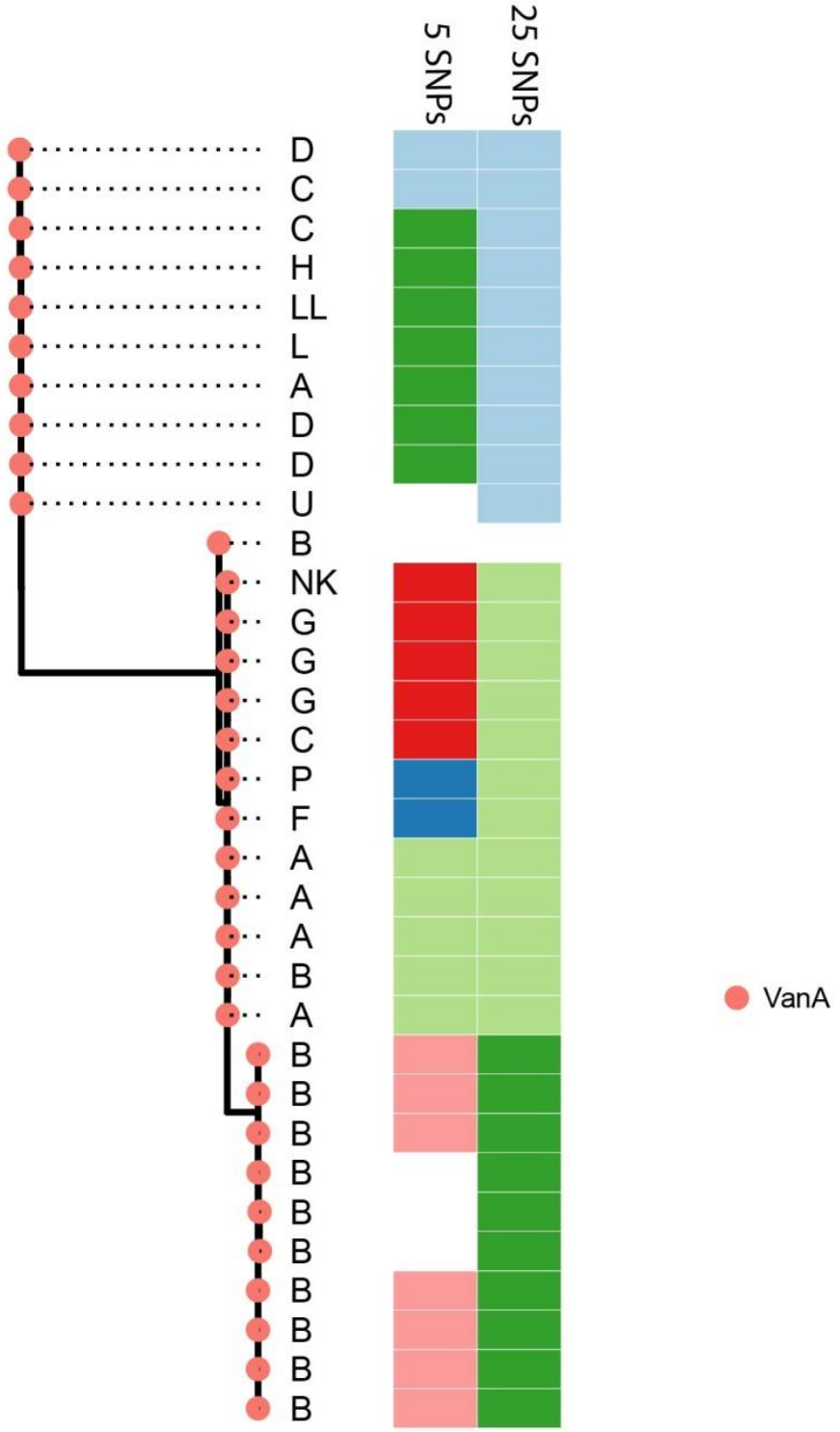

Figure 7. MLST 1424 *van*A VREfm annotated by healthcare facility (HCF) code, displaying clusters at the 5 and 25 SNP level. Letters indicate HCF code. Coloured bars indicate clustering of isolates.

Among MLST 796 VREfm isolates, there were six clusters at the 25 SNP level and 17 clusters identified at the 5 SNP level. The largest cluster identified, at the 25 SNP level, comprised 160 of 183 isolates (87%) (Figure 8). The large cluster at the 25 SNP level incorporated 31 of 40 HCF (77.5%).

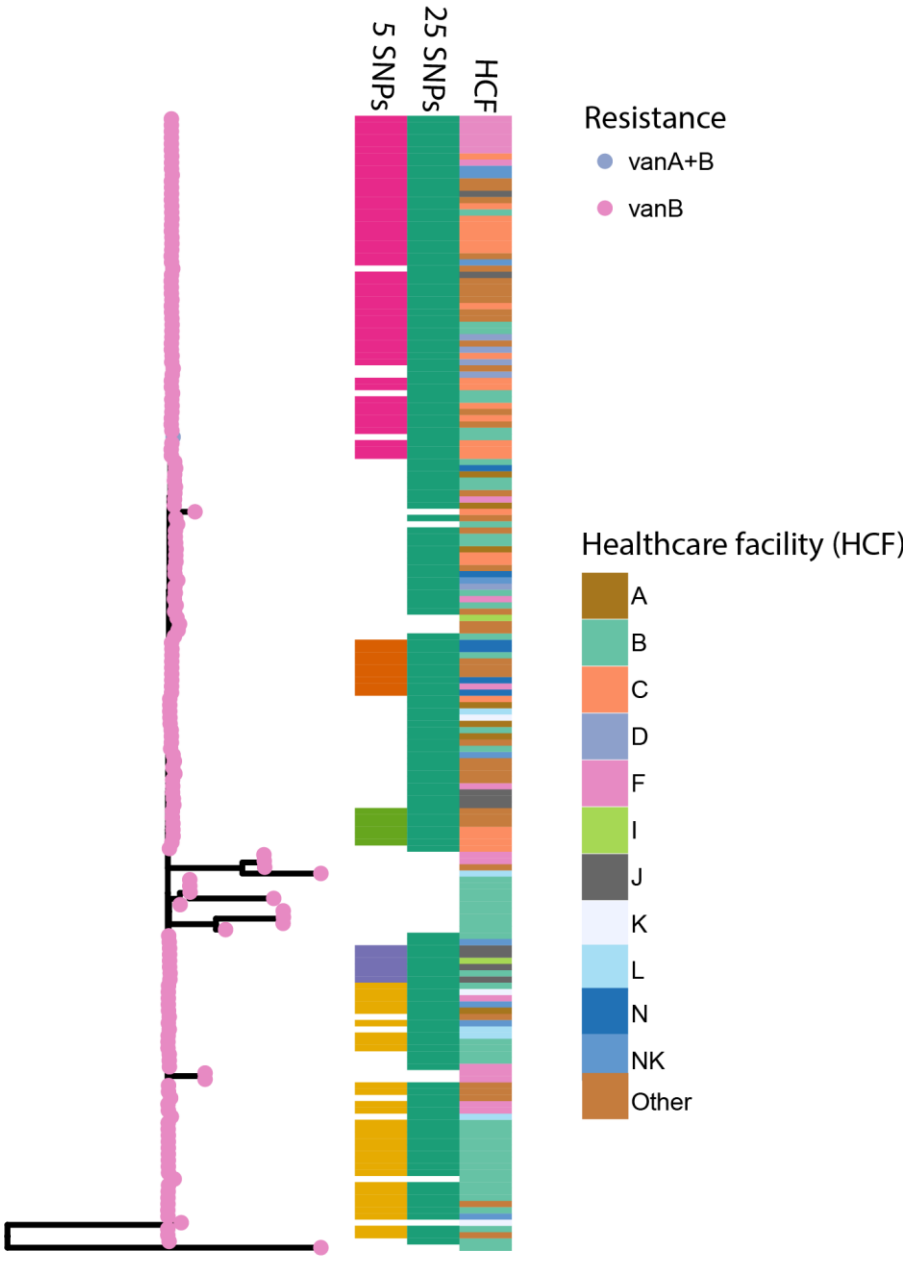

Figure 8. MLST 796 VREfm tree annotated by healthcare facility (HCF) code, displaying linkage clusters at the 5 and 25 SNP level. Only HCF with more than 10 isolates shown. Five SNP clusters only shown where six or more isolates included in the cluster.

5.4.1 Pairwise SNP differences

One method of assessing the possibility of transmission of VREfm occurring is to examine the diversity of pairwise SNP differences among isolates originating from within a facility or from

two different facilities. When comparing two groups of isolates, the group with a lower number of pairwise SNP differences has a lower level of genetic diversity. Furthermore, a lower number of pairwise SNP differences could indicate transmission of isolates, as genetic diversity is expected to decrease with transmission and mixing of genetic material between isolates.

vanA VREfm

The median number of pairwise SNP differences within *van*A VREfm isolates was higher than the median number found among *van*B VREfm isolates (p<0.001). Among *van*A VREfm, the median number of pairwise SNPs was higher between HCF than within HCF (Figure 11, Appendix 4).

vanB VREfm

Among *van*B VREfm, the median number of pairwise SNP differences was higher among *van*B excluding MLST 796 compared to *van*B 796 only (p<0.001) (Figure 11, Appendix 4). Among *van*B VREfm, the median number of pairwise SNP differences was higher within HCF compared to between HCF. For *van*B VREfm 796 only, the median number of SNPs within HCF was higher than that between HCF.

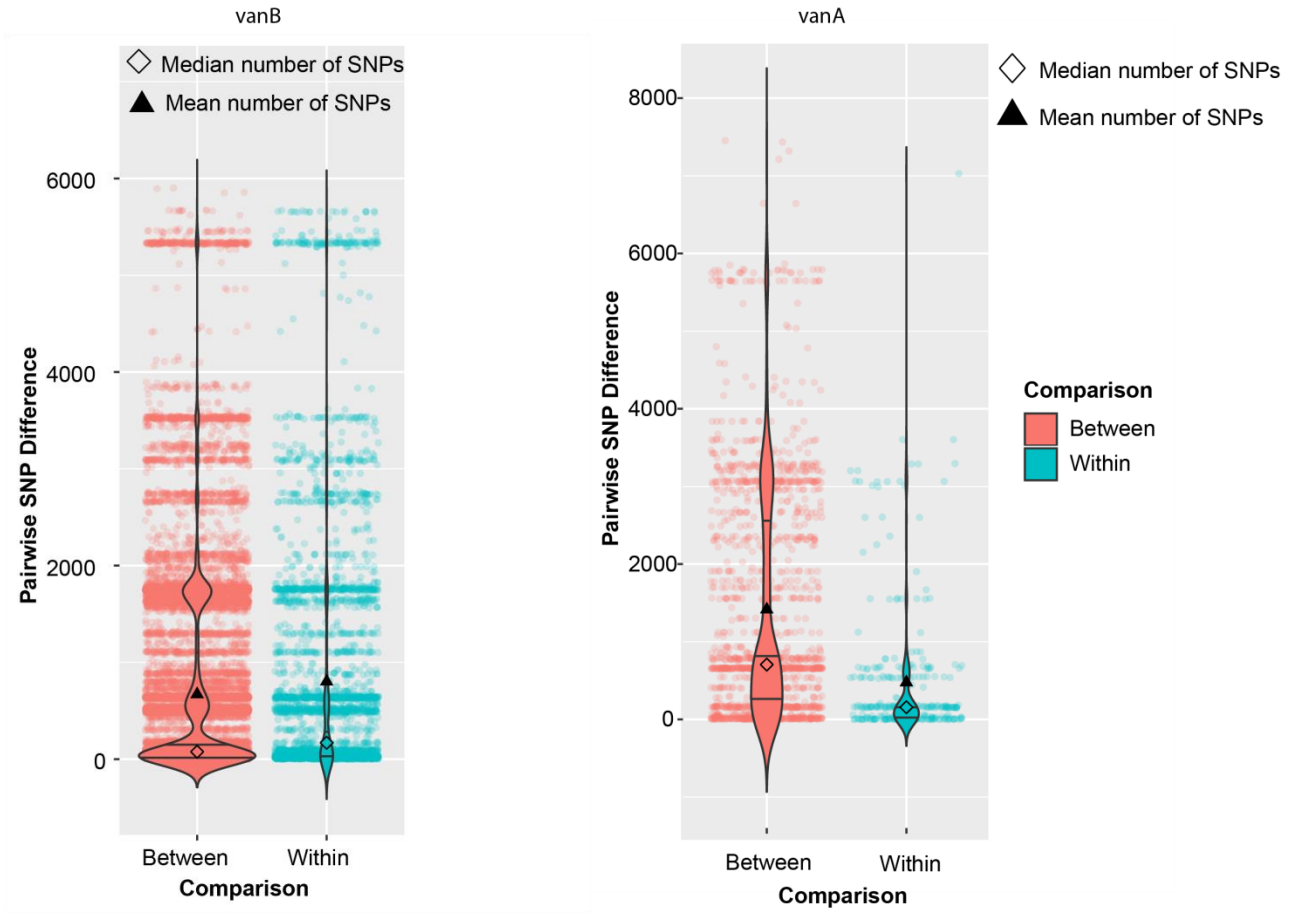

Figure 9. Comparison of pairwise SNP differences for *van*B and *vanA* for isolates originating from within and between healthcare facilities (HCF). Where two isolates have originated from two different HCF, they have been classified as "between HCF." Where two isolates have originated from the same HCF, they have been classified as "within HCF".

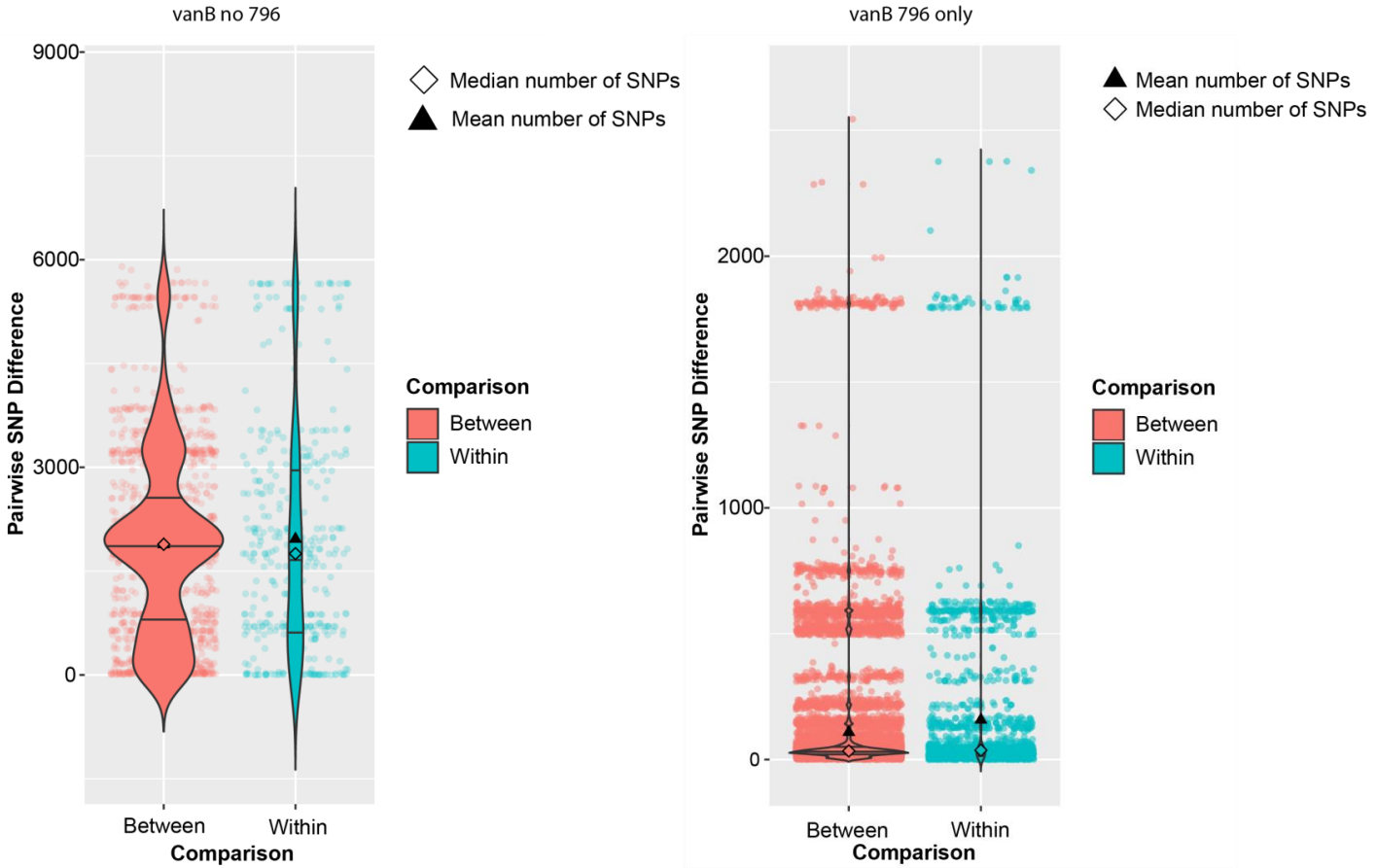

Figure 10. Comparison of pairwise SNP differences for *van*B excluding MLST 796 and for MLST 796 *van*B only for isolates originating from within and between healthcare facilities (HCF). Where two isolates have originated from two different HCF, they have been classified as "between HCF." Where two isolates have originated from the same HCF, they have been classified as "within HCF".

6. Discussion

This study assessed the prevalence and genomic diversity of VREfm from all specimens, and VSEfm from blood cultures, over a one-month period in 2018 in Victoria. There has been no significant increase in the incidence of VREfm clinical infection since a similar cross sectional survey conducted in 2015, nor has there been an increase in the incidence of VREfm colonisation or clinical infection for *van*A isolates. While the overall proportion of *van*A VREfm has not increased between 2015 and 2018, the presence of a new MLST among *van*A VREfm was identified, MLST 1424. In addition, multiple clusters of isolates were identified, indicating that transmission of VREfm is likely to be occurring within and between HCF in Victoria.

While annual reports from the Australian Group on Antimicrobial Resistance (AGAR) reported that the proportion of VREfm with *van*A has increased in Victoria, the increase in Victoria is not statistically significant, consistent with our results (36). Of note, this study has identified MLST 1424 *van*A VREfm, which was not identified in the 2015 study. AGAR sentinel surveillance of blood cultures identified a single 1424 isolate in Victoria in 2017, and two isolates in 2018, however it was not reported in Victoria prior to this (19, 47-49). In 2016, AGAR identified 1424 in NSW (19). In 2018, AGAR identified 1424 in all jurisdictions except for the Northern Territory and Western Australia, which could indicate that this MLST is spreading throughout Australia (49). In addition, in 2017, 1424 was isolated in Scotland, indicating possible international spread (50).

MLST 1424 comprised the majority of *van*A VREfm isolates in this study. Among 1424 *van*A VREfm, there were three clusters containing potentially related isolates. Some clusters of MLST 1424 were more closely associated with other MLSTs. This reinforces why WGS is useful for more accurate identification of transmission networks. Following application of WGS, MLSTs do not always cluster together, demonstrating the improved accuracy of WGS for defining linked isolates.

MLST 1424 lacks one of the loci, *pstS*, one of the housekeeping genes on which the MLST scheme is based. *E. faecium* which lack this locus are known as *pstS-*, and include both MLST 1421 and 1424. *PstS- E. faecium* was first isolated in Australia in 2012 and the emergence of these sequence types is thought to be contributing to the national increase in *van*A VREfm (51). It has already been suggested that these isolates may be part of a multi-jurisdictional outbreak and they have been shown to have spread between Victoria and NSW (51, 52). In addition, the emergence of MLST 1421 and 1424 in recent years, both missing the *pst* allele,

suggests that MLST may become increasingly limited in its usefulness for the surveillance of VREfm, and that WGS is a more appropriate method (50, 51).

The majority of VREfm identified within this study were ML ST 796 *van*B isolates, consistent with the 2015 study and AGAR sentinel surveillance data (47). Not only is this MLST found in most HCFs, it caused most of VREfm bacteraemia identified in this study. The limited number of pairwise SNP differences among MLST 796 indicates a low level of genetic diversity compared to other *van*B MLSTs. This low level of genetic diversity is likely to be a consequence of multiple episodes of transmission of 796 within and between HCF. Providing further evidence to the widespread transmission of *van*B MLST 796, 87% of isolates in this study fell within a large cluster as defined at the 25 SNP level.

The majority of VREfm in this study were isolated from specimens collected for screening purposes. While there was no significant difference in the proportion of *van*A VREfm identified by screening compared to urine and clinical specimens, HCF B, which conducted increased screening as part of a point prevalence screen for the duration of the study, identified the majority of VREfm isolates submitted. The majority of 1424 isolates were isolated from screening specimens collected by HCF B. In addition, HCF B identified most of the patients in this study with VREfm with more than one *van* genotype and MLST and identified all seven of the most prevalent sequence types in the study. This could indicate that increased screening may capture a broader diversity of MLSTs, which is important for the identification of emerging sequence types and changes in the genomic epidemiology of VREfm.

VREfm was distributed across many HCF. There were multiple clusters within all MLSTs analysed, which could indicate transmission both within and between Victorian HCF. Among *van*A VREfm, there was a higher level of median pairwise SNP differences from isolates between HCF compared to within HCF. This could indicate that the population of *van*A VREfm circulating within HCF have less genetic diversity than those between HCF. Therefore, it is likely that transmission of *van*A VREfm between HCF is currently limited. Conversely, among *van*B VREfm, there was a lower level of median pairwise SNP differences between HCF compared to within HCF. This could indicate that transmission of *van*B VREfm between HCF is more widespread than *van*A VREfm, as isolates between HCF are less genetically diverse than those circulating within individual HCFs.

Among *van*B VREfm, MLST 796 displayed a lower level of genetic diversity, as indicated by a relatively low number of pairwise SNP differences. This is consistent with this MLST being widespread throughout HCF in Victoria. Control of VREfm, by active surveillance screening, isolation of patients, and identifying patient contacts and transmission pathways, is resource

intensive. Future surveillance efforts need to prioritised, potentially focusing on preventing the spread of *van*A VREfm, and in particular, the spread of new MLSTs such as 1424.

6.1 Limitations

This study had several limitations including a lack of standardised screening practices in Victorian HCF and insufficient epidemiological data to confirm putative transmission pathways. In addition, the effect of recombination on the genomics of VREfm has not yet been accounted for.

At the time of this study, VREfm was not notifiable in Victoria, therefore surveillance and response was the responsibility of individual HCFs. In Victoria, there is currently no standardised screening or management guidelines for VRE (9). It is likely that varied screening practices result in several colonised patients not being identified, which contributes to the spread of VREfm within and between facilities. Variation in screening practices between HCFs also means it is likely that the numbers of VREfm identified within colonised patients have been underestimated by this study.

Community patients in this study were defined as those where the healthcare origin was unknown. In reality, detailed healthcare history would be required to accurately classify patients as a community source of VREfm. Community isolates are usually defined as originating from patients with samples collected within 48 hours of admission to hospital (49). It is therefore possible that community patients have been misclassified. Furthermore, this study was not able to collect data on patient admissions or movements within and between HCFs in order to further identify putative transmission pathways. Future studies should endeavour to collect this information and combine it with genomic analyses to inform the required public health response. This is likely to be more feasible if *van*A VREfm becomes notifiable under the *Victorian Public Health and Wellbeing Regulations 2019.*

As this was a laboratory based surveillance study, the clinical significance of isolates originating from urine samples was not always clear. As multi-resistant organisms can colonise the urinary tract, it is possible that many isolates from urine samples represent colonisation rather than infection. More detailed medical epidemiological data would be required to accurately classify these samples, as this information is often not provided on laboratory request forms, and needs to be obtained from the treating clinician. If isolates from urine samples represent colonisation, this study may have overestimated the true prevalence of VREfm infection. However, patients with urine colonised by VREfm still represent a possible source for transmission to the healthcare environment and other patients.

This study identified clusters based on SNP cut-off levels which are somewhat arbitrarily defined. Analyses have not yet accounted for the presence of recombination within VREfm, as this was considered beyond the scope of the project within the MAE context. This means that loci classified as an MLST may be reclassified once the effect of recombination is included (15). Recombination can also artificially inflate the number of SNPs (53). Further analyses will account for recombination in addition to conducting phylogenetic comparisons to isolates from the 2015 study. This may reveal additional clusters not identified in this analysis.

6.2 Further work

As mentioned, it is feasible that increased screening such as that being conducted by HCF B is able to identify a higher number of VREfm isolates and a wider diversity of MLSTs. To determine this in relation to specific facilities, additional information including the details of facility screening protocols for VREfm and the patient load of each facility are required. This could have implications for future screening practices, which could be based on the patient load of each facility, and how screening could be used as part of state-wide surveillance.

As plasmids carried by VREfm can play an important role in transmission of *van* genes, additional analyses will also assess the role of VREfm plasmids in transmission. The use of a core genome approach to analyses means that the accessory genome (the part not shared by all isolates in this study) was not assessed (29). Further work may also include assessing the pan-genome (complete genome) and how this relates to the epidemiology of VREfm in Victoria.

7. Conclusions

While this study has shown that the proportion of *van*A VREfm have not increased in Victoria between the two study periods, 2015 and 2018, a new MLST was identified. This study has also demonstrated the potential use of WGS for future surveillance of VREfm in Victoria. WGS enabled the identification of several putative transmission events within and between Victorian HCF. These findings indicate that ongoing surveillance of VREfm, particularly *van*A VREfm, is required, in order to inform appropriate public health measures to minimise further spread.

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typeable vancomycin-resistant *Enterococcus faecium*. *J Antimicrob Chemother*. 2016; 71:3367-71.

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Appendix 1. VREfm phylogenetic trees

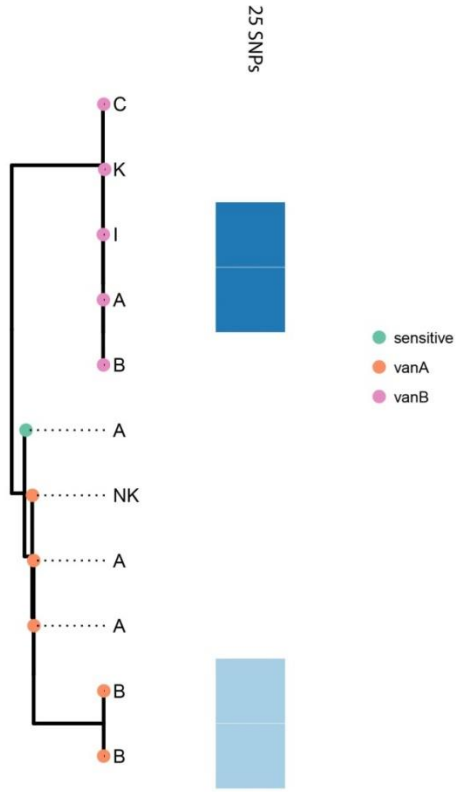


Figure 11. MLST 203 *E. faecium* tree annotated by HCF code, displaying clusters at the 25 SNP level. Letters indicate HCF code, NK indicates no known HCF.

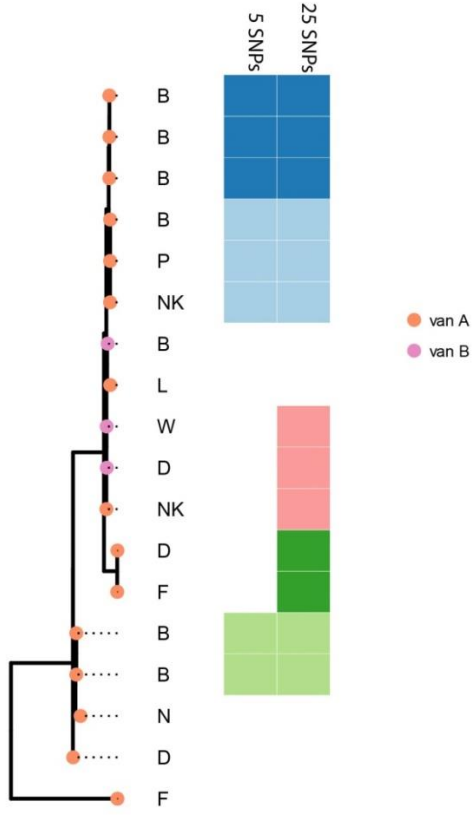


Figure 12. MLST 1421 *E. faecium* tree annotated by HCF code, displaying clusters at the 5 and 25 SNP level. Letter indicate HCF code, NK indicates no known HCF.

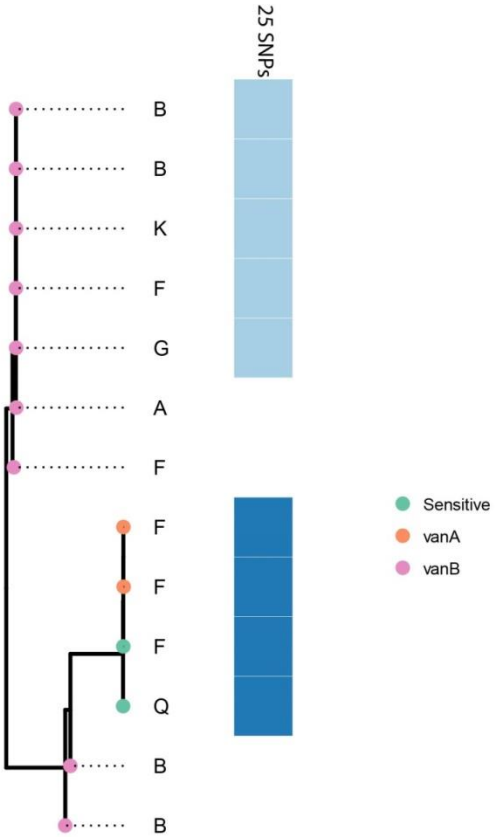


Figure 13. MLST 17 *E. faecium* tree annotated by HCF code, displaying clusters at the 25 SNP level. Letter indicate HCF code.

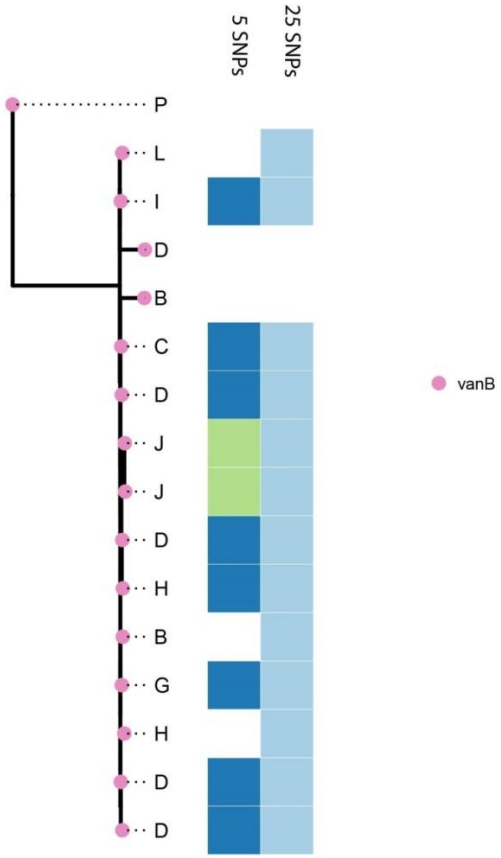


Figure 14. MLST 78 *E. faecium* tree annotated by HCF code, displaying clusters at the 5 and 25 SNP level. Letter indicate HCF code.

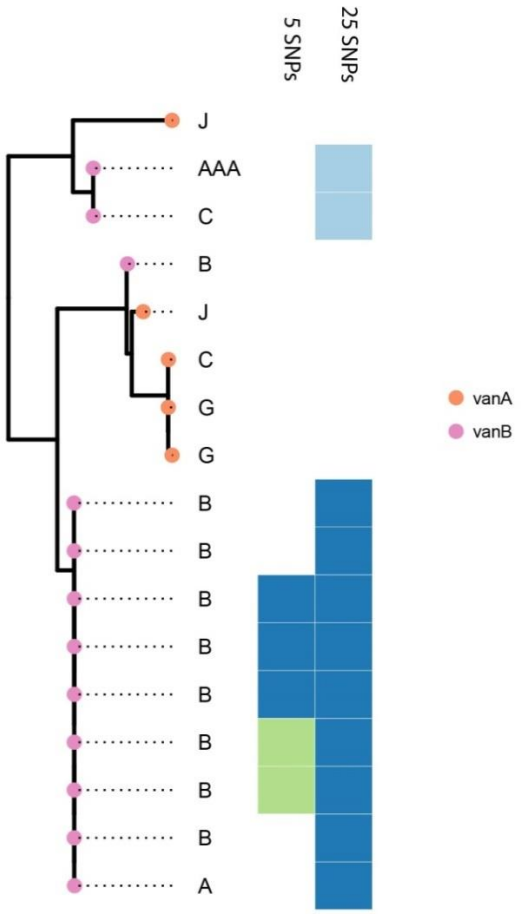


Figure 15. MLST 80 *E. faecium* tree annotated by HCF code, displaying clusters at the 5 and 25 SNP level. Letter indicate HCF code.

Appendix 2.

Patients with more than one VREfm MLST or *van* gene

Patient identification number	Sample type	*Van* gene	MLST	Healthcare facility	Interval
2	Screening – rectal swab Screening – rectal swab	*van*B *van*A	Not typeable 1424	B	Collected on the same day
1	Screening – rectal swab Screening – rectal swab	*van*B *van*A	1283 203	B	Collected 1 day apart
15	Screening – rectal swab Screening – rectal swab	*van*B *van*B	796 80	B	Collected 10 days apart
29	Screening – rectal swab Clinical – peritoneal cavity fluid	*van*B *van*B	796 Novel 1	C	Collected 1 day apart
49	Screening – rectal swab Screening – rectal swab	*van*B *van*B	796 992	B	Collected 10 days apart
56	Urine Urine	*van*A *van*B	1424 78	L	Collected on the same day
58	Screening – rectal swab Screening – rectal swab	*van*B *van*A	Novel 2 1424	B	Collected on the same day

Appendix 3.

Pairwise SNP distribution among *van* genotypes and within and between healthcare facilities (HCF)

Van genotype	Median no. and range of pairwise SNP differences	Difference in distribution between and within HCF	More diversity within or between HCF?
***van*A**	659 SNPs, range 0–7453	-	Between
Within HCF	157 SNPs, range 0–7028	$p<0.001$	
Between HCF	706 SNPs, range 0–7453		
***van*B**	83 SNPs, range 0–5902	-	Within
Within HCF	169 SNPs, range 0–5671	$p<0.001$	
Between HCF	76 SNPs, range 0–5902		
***van*B MLST 796 only**	29 SNPs, range 0–2231	-	Within
Within HCF	31 SNPs, range 0–2226	$p<0.001$	
Between HCF	29 SNPs, range 0–2231		
***van*B excluding MLST 796**	1993 SNPs, range 0–5902	-	No difference $p=0.17$
Within HCF	1754 SNPs, range 0–5671	$p=0.40$	
Between HCF	2021 SNPs, range 5–5902		

Appendix 4. Vancomycin-resistant Enterococci (VRE) Patient Information Sheet

What is VRE?

Enterococci are bacteria which are normally found in the bowel of both humans and animals. Vancomycin is an antibiotic which is usually used to treat these bacteria. Some enterococci have developed resistance to vancomycin, therefore this antibiotic can no longer be used to treat these infections. These bacteria are called vancomycin-resistant enterococci or VRE.

Causes of VRE

Enterococcal bacteria can develop resistance to vancomycin in your bowel after you have taken broad-spectrum antibiotics. VRE can be spread by coming into contact with a person who has VRE, as the bacteria can be shed in the person's faeces or on their skin.

Most people who have VRE are colonised, meaning they have the bacteria in their bowel but do not show any symptoms of illness. Infections caused by VRE occur mostly in people who are already unwell or who have a poorly functioning immune system. People who carry VRE have a risk of developing an infection if they undergo treatment in an intensive care unit, receive haemodialysis or cancer treatment. Taking antibiotics can also increase your risk of developing an infection if you are already colonised by VRE.

Diagnosis of VRE

VRE may be diagnosed by your doctor if you develop an infection, such as an infected wound, urinary tract infection, or an infection of the blood. Alternatively, if you are admitted to hospital for another reason, you may be screened for the presence of VRE. Screening is performed to prevent VRE is from spreading between patients within a hospital.

Hospitals often screen people who have previously been admitted to other hospitals for treatment, and those who have had known contact with other people who have had VRE in the past. Some hospitals screen all patients admitted to wards where this is a known risk of VRE, such as the intensive care unit, or oncology ward.

The most common and preferred method for screening of VRE is to test a rectal swab or a sample of faeces. Swabs may also be taken from any open wounds or from catheters or cannulas. The test result from the screening sample will usually take one or two days to be received.

Stopping the spread of VRE

As a precaution, patients suspected of having VRE may be placed in a separate hospital room, to prevent the bacteria spreading to other patients. Staff who come into contact with patients suspected of having VRE wear gowns and gloves to ensure they do not spread the bacteria to other patients. Any visitors need to clean and disinfect their hands before entering or leaving the room to prevent them contracting the bacteria.

Treatment of VRE

If you are diagnosed with VRE, your doctor will decide if you need any treatment. As many people carry VRE without displaying any symptoms of illness, you may not need any treatment. As part of future hospital admissions, you may be retested for VRE, as it is not clear how long people carry the bacteria for.

Appendix 5. Presentation at AEA 2019

A population level genomic snapshot study of Vancomycin-Resistant Enterococci, Victoria, November 2018

Sophie Bowman-Derrick
Masters of Applied Epidemiology Scholar

Doherty Institute | Australian National University | Victoria State Government Health and Human Services

What is VRE?

- Enterococci are normal flora of the gastrointestinal tract
- Intrinsic or acquired antimicrobial resistance
- Vancomycin-resistant Enterococci

Public health importance

Source: Scimex.org, 2018

Source: Centers for Disease Control and Prevention

- Higher morbidity and mortality
- More difficult to treat
- Increased healthcare costs
- Difficult to control

Van genes

VanA
- Highly resistant to vancomycin
- Can be resistant to teicoplanin
- Until recently, little evidence of *van*A VRE in Australia

VanB
- Mostly resistant to vancomycin only
- considered to be endemic in Australia

AGAR

Surveillance in Australia conducted by AGAR
- Blood isolates only
- In Victoria, only five large healthcare facilities contribute

2018 Victorian VRE snapshot objectives

- To investigate changes in the prevalence and genomic diversity of VRE
- To determine if *van*A VRE are increasing
- To evaluate the presence of transmission between healthcare facilities

Methods

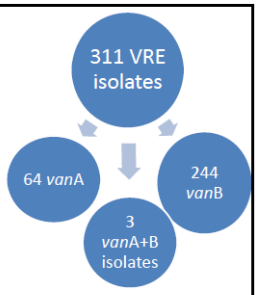
All Victorian labs asked to submit all VRE from any sample, 01–30 November 2018

All isolates underwent whole genome sequencing

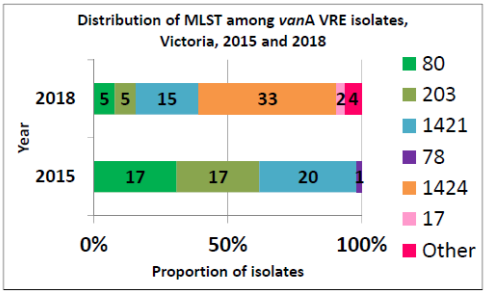
Bioinformatics: identification of *van* genes, **multi-locus sequence type (MLST),** and phylogenetic analyses

Results

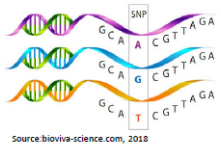
- Proportion of *van*A:
  - 19% 2015
  - 21% 2018
- Incidence of VRE clinical infection:
- **2015:** 0.15 per 100,000 population per month
- **2018:** 0.16 per 100,000 population per month

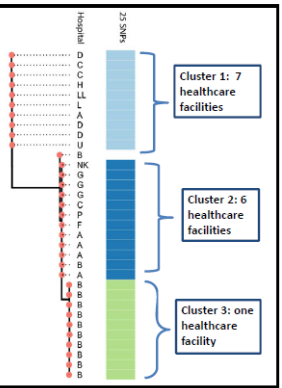


New MLST among *van*A VRE in Victoria



Identification of putative transmission pathways among MLST 1424





Conclusions

- No significant increase in VRE infection, or vanA VRE, between 2015 and 2018 in Victoria
- MLST 1424 identified, not previously seen in Victoria
- Transmission of VRE likely occurring between healthcare facilities
- Intermittent cross-sectional studies can be used for the surveillance of health-care acquired pathogens

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Dr Norelle Sherry
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Marion Easton
Janet Strachan

Dr Emma Field

Chapter V: Salmonellosis at a Mother's Day Lunch in Regional Victoria

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1. Preface

In May 2018, the Communicable Diseases, Prevention and Control (CDPC) branch at the Victorian Government DHHS was notified of an outbreak by a regional council. The council received notification of a possible outbreak by a restaurant who had received several complaints from patrons. The patrons had developed a gastrointestinal illness following attendance at a Mother's Day function held at the restaurant. Follow up of the initial complaints led to the identification of a food-borne outbreak. Dr Zoe Cutcher, an OzFoodNet epidemiologist at DHHS, assigned me to the outbreak investigation as it appeared to be an ideal scenario for me to apply my knowledge and meet this core competency for the MAE.

1.1 My role

I was a co-investigator in this outbreak, working in a team that included Senior Public Health Officer, Sally Atkinson. Several other Public Health Officers (PHOs) assisted with the interviewing of function attendees. Dr Zoe Cutcher provided me with advice on the design of a menu-based questionnaire and provided me with guidance on data analysis using Stata. Sally Atkinson was responsible for liaising with the regional council and the regional Environmental Health Officer (EHO). The regional EHO obtained booking lists, details of menu items, and food processes from the restaurant and conducted interviews of restaurant staff.

My role in the investigation included:

- Designing the structured outbreak investigation questionnaire
- Interviewing a proportion of the function attendees
- Entering completed questionnaires into the DHHS Public Health Event Surveillance System (PHESS)
- Identifying notified cases with *Salmonella* species sharing Multiple-locus Variable Analysis of tandem repeats (MLVA) patterns similar to the outbreak strain, in the month prior to the outbreak
- Conducting three day food history interviews for notifications with MLVA patterns that were similar to the outbreak strain
- Attending an inspection of the egg farm supplying the restaurant
- Extraction of questionnaire data into Microsoft Excel and analysis of responses using Stata
- Write up of the epidemiological section of the outbreak report which was distributed to the regional council

1.2 Lessons learnt

My involvement in this investigation enabled me to apply classroom skills and theory to a real-world setting. I learned a number of invaluable skills that will be applied to future outbreak investigations. Practical skills obtained during this investigation include the design of a structured outbreak questionnaire, data entry into PHESS, linking individual cases to an outbreak event within PHESS, extracting data from PHESS into Microsoft Excel, and analysis of outbreak data in Stata.

My experience of this investigation highlighted the importance of a teamwork approach, even in relatively small outbreaks. Having assistance from multiple PHOs, epidemiologists and EHOs ensured that the investigation was conducted as quickly as possible. I also encountered some of the difficulties of working with regional EHOs where communication is mostly by telephone. As the initial staff interviews and food processes information did not include adequate detail, several phone conversations with the regional EHOs and a second restaurant inspection were required to obtain additional information. For the next food-borne outbreak investigation I am involved in, I would consider holding a brief teleconference with council staff prior to the restaurant inspection in order to ensure clarity regarding the information and level of detail required.

This investigation enhanced my understanding of the importance of pursuing multiple lines of evidence during an outbreak. Although the investigation was conducted in a timely manner, all food and environmental samples tested were negative for *Salmonella.* Despite this, epidemiological investigation combined with the detailed interrogation of food processes at the restaurant provided strong evidence for the suspected food vehicle involved in this outbreak.

I gained experience in the use of MLVA to screen for additional cases of salmonellosis which may not have been initially apparent in the investigation. While the use of MLVA patterns did not identify any additional cases associated with this outbreak, this process demonstrated the importance of active case-finding. My involvement in this investigation also demonstrated the concept of undercount in relation to surveillance systems, as the interviewing process enabled the identification of cases of salmonellosis, which would not otherwise have been notified to the DHHS.

The site visit to the egg farm was an invaluable experience. Despite my training as a veterinarian, prior to this investigation I had minimal understanding of the processes involved in egg production. I am now much more aware of the complexities of trace-back investigations, egg

safety, and pathogen transmission in food-producing animals. The egg farm visit also provided me with insight into the food supplier business's perspective in an outbreak investigation.

1.3 Public health implications

This outbreak investigation provided opportunities to educate restaurant staff on safe food preparation, particularly for egg-based foods. As a result of the outbreak investigation, the regional council recommended that the restaurant use processed egg products, rather than fresh eggs in the future. The egg farm inspection enabled the Principal Veterinary Officer to educate the owner of the facility on additional methods to minimise the risk of *Salmonella* in poultry and eggs. These measures are expected to reduce the risk of salmonellosis outbreaks occurring as a result of the preparation of egg-based foods at this restaurant in the future. In addition, the case interview process provided an opportunity to educate function attendees regarding the risks associated with eating food containing lightly cooked eggs.

1.4 Acknowledgements

I acknowledge the following people for their assistance with this project:

- Dr Zoe Cutcher, OzFoodNet Epidemiologist, for involving me in this investigation, teaching me how to design a menu-based questionnaire, and inviting me to attend the site visit at the egg farm
- Marion Easton for showing me how to screen for additional outbreak cases of *Salmonella* based on MLVA patterns and for extracting the relevant data from PHESS
- Dr Emma Field for providing useful feedback on my writing and data analysis
- Siobhan St George for teaching me how to construct a working Stata do-file and to recode data for Stata analysis
- PHOs Sally Atkinson, Matthew De Souza, Dania Thakar, Sarah Cleghorn and Kay Sturge for assisting with case interviews and patiently answering my questions
- The restaurant staff for participating in interviews and environmental investigation and for providing function contact lists
- The egg supplier for allowing me to participate in the site visit
- Dr Joni Segal, Principal Veterinary Officer, for allowing me to participate in the site visit and for sharing his wealth of knowledge
- All people who participated in questionnaires conducted as part of this investigation

2. Abstract

Introduction: In May 2018 a *Salmonella* Typhimurium MLVA 03-15-11-10-523 outbreak occurred following a function held at a restaurant in Victoria. Epidemiological and environmental investigations were performed with the aim of determining the source of the outbreak.

Methods: A retrospective cohort study was conducted. Function attendees were interviewed using a structured menu-based questionnaire. Descriptive analysis of the cohort was performed and the risk ratio of illness (RR) associated with the consumption of each menu item was calculated. Initial microbiological testing of faecal samples was conducted by either Victorian diagnostic laboratories or MDU. MDU performed MLVA on all *S.* Typhimurium isolates.

Results: Thirty-nine out of 53 function attendees (73.6%) were interviewed. Nineteen cases were identified resulting in an attack rate of 48.7%. There was a significant association between consumption of chocolate mousse and illness (RR 5.1, 95% confidence interval: 1.8–14.6) indicating that this was the most likely food vehicle for *Salmonella.* The chocolate mousse was prepared using lightly cooked eggs.

Conclusion: Chocolate mousse was the most likely food source for this outbreak. The use of lightly cooked eggs is likely to have contributed to contamination with *Salmonella*. Using appropriate cooking temperatures is essential when preparing eggs in order to minimise the risk of food-borne salmonellosis.

3. Background

Salmonella is a common cause of food-borne gastroenteritis (1). In addition to transmission through contaminated food, *Salmonella* can be spread through contact with the faeces of a person or animal that is infected with or carrying the bacteria (2). Salmonellosis can be severe with symptoms including fever, vomiting, and diarrhoea which may be bloody (3). Hospitalisation may be required due to dehydration or sepsis (3). Worldwide, non-typhoidal Annually, more than 90 million cases of gastroenteritis and 155,000 deaths are caused by *Salmonella* (4, 5).

In Australia, the rate of *Salmonella* notifications has increased over the past five years (6). *Salmonella* is a significant cause of a large number of food-borne outbreaks (1, 7, 8). In 2017, there were over 16,000 notifications of salmonellosis in Australia, a rate of 67 per 100,000 population per year (6). Salmonellosis is often associated with the consumption of foods containing undercooked egg (9). In 2011, OzFoodNet reported that almost half of *Salmonella* outbreaks were associated with eggs (8).

There are more than 2,500 serotypes of *Salmonella* (3, 4, 10). *Salmonella enterica* serovar Typhimurium (hereafter referred to as *S.* Typhimurium) is the most commonly notified serotype and a common cause of salmonellosis outbreaks in Australia (8). *S.* Typhimurium was the causative agent of more than a quarter of Australian food-borne outbreaks from 2007-2011 (1, 7, 8, 11-13). Eggs have caused many previous food-borne outbreaks of *S.* Typhimurium in Australia and internationally (14). From 2001-2011, more than 150 egg-associated outbreaks in Australia were caused by *S.* Typhimurium; this represented 90% of egg-associated outbreaks, caused 2,880 cases of illness, and more than 500 hospitalisations (15).

While the infectious dose of *Salmonella* is low in humans, poultry may harbour *Salmonella* while displaying no signs of infection (16). Eggs can become contaminated with *Salmonella* by transmission from the chicken's reproductive tract, or the bacteria can cross the eggshell during or after laying (17). Horizontal transmission, whereby *Salmonella* crosses the eggshell, is the most common way in which eggs become contaminated with *S.* Typhimurium (17, 18).

Salmonella present on egg shells may contaminate the internal egg contents or other food during preparation (19). While the reported frequency of eggs contaminated with *Salmonella* is low, food-borne illness can occur when contaminated eggs are eaten raw or are only lightly cooked (20). As consumers are increasingly eating raw or lightly cooked foods, undercooked egg is of particular concern as a potential vehicle for salmonellosis (16).

In Victoria, all *Salmonella* isolates are sent to the MDU for further characterisation by subtyping. One method of typing is MLVA which is used for various species of *Salmonella* including *S.* Typhimurium (21). MLVA assesses the number of tandem repeats, which are short repetitive sequences of DNA, at rapidly evolving loci or sites of the bacterial genome (10, 22). Between isolates, the number of tandem repeats at each locus differs (12). The resulting MLVA pattern is a code indicating the number of repeats at each loci (23). MLVA is used to enable the detection of clusters of isolates and can be used to assist in determining causality and conducting trace-back investigations (10, 22).

Following a Mother's day function held on 13 May 2018, a restaurant received several complaints from function attendees who had become ill with gastroenteritis. The restaurant notified their local council regarding a possible food-borne outbreak. On 21 May 2018, the council notified CDPC. As the initial notification included reports of several people with gastroenteritis following attendance of the restaurant, an outbreak was identified. An outbreak investigation was conducted to identify the most likely source of illness. The investigation also aimed to identify the need for public health action to prevent the occurrence of additional cases.

4. Methods

A retrospective cohort study of the function attendees was conducted. Environmental investigation included a restaurant inspection, staff interviews, and a site visit of the egg supplier. Laboratory investigation included testing of faecal samples from cases, food and environmental samples from the restaurant, and environmental samples from the egg supplier.

4.1 Epidemiological investigation

4.1.1 Study population

Booking lists were obtained from the restaurant in order to identify all function attendees. The restaurant had not received any reports of illness from patrons who had attended in the days prior or following the function, and there were no booking lists available for these days. The cohort initially included any person who had attended and eaten at the restaurant on Sunday 13 May. The Mother's day function comprised three sittings – breakfast, early lunch (11:00) and late lunch (14:00). Once it was determined that none of the 28 people who had eaten breakfast on the day had become ill, the cohort was refined to include only those who had eaten lunch at either the 11:00 or 14:00 sittings. The early lunch sitting included 28 diners and the late lunch sitting included 25 diners. The menu for both lunch sittings was identical, therefore diners in

these sittings were considered to be one cohort for analysis. The cohort was therefore defined as the 53 people who ate lunch at the restaurant on 13 May.

4.1.2 Questionnaire design and interview of function attendees

A structured outbreak investigation questionnaire was designed based on the set menu provided by the restaurant. The details of the function menu items were clarified with the restaurant via the local EHO. Attempts were made to interview all 53 people in the cohort by telephone. Function attendees that reported becoming ill after eating at the restaurant were asked to provide a faecal sample for laboratory testing if they had not already provided one as advised by their doctor. On completion of the interview, all function attendees were educated regarding the risks associated with eating lightly cooked eggs.

Two case definitions were developed (Box 1). Probable cases included anyone who ate at the restaurant on 13 May 2018 and subsequently developed gastroenteritis. To allow for staff who developed gastroenteritis following the function, the probable case definition also includes anyone directly involved in food preparation for the function. Confirmed cases included anyone who ate at the restaurant on 13 May, or was directly involved in food preparation for the function on the 13 May, subsequently developed gastroenteritis, and had provided a faecal sample that tested positive for *S.* Typhimurium. Once the MLVA pattern for the *S.* Typhimurium was determined, the confirmed case definition was revised to include the MLVA result.

Box 1: Outbreak case definitions

Confirmed case

A person who ate at the restaurant on 13 May, 2018, or was directly involved in food preparation for the function held on 13 May, subsequently developed gastroenteritis, and had a faecal sample positive for *S.* Typhimurium MLVA 03-15-11-10-523.

Probable case

A person who ate at the restaurant on 13 May, 2018, or was directly involved in food preparation for the function held on 13 May and subsequently developed gastroenteritis with symptoms including diarrhoea, abdominal pain, vomiting and/or nausea within 72 hours of attending the function, but did not have a faecal specimen tested by a laboratory.

4.1.3 Statistical analysis

On completion of all interviews, responses were entered into the question package created in the DHHS notification database, PHESS. Data were then extracted into Microsoft Excel and analysed in Stata® IC 15.1 (StataCorp, Texas, USA). An epidemic curve was constructed which included both function attendee cases and staff cases. Descriptive analysis of cases was performed to characterise the demographics and symptoms. Univariate analysis was conducted to calculate the crude risk ratio for each menu item, with 95% confidence intervals (CI). For menu items where the number of exposures was too small (i.e. an exposure of zero was present), an exact logistic regression was used to calculate an adjusted odds ratio, with 95% CI.

4.2 Environmental investigation

4.2.1 Restaurant inspection and interrogation of food preparation

An inspection of the restaurant was conducted on 18 May in accordance with the *Victorian Guidelines for the investigation of gastrointestinal illness*, using the *Gastroenteritis Outbreak Onsite Assessment form* (24). The inspection included interviewing staff, collecting food samples, and reviewing food processes, hygiene and cleaning practices in the kitchen. Ill staff were asked about food consumed in the three days prior to onset of illness and were requested to provide faecal samples for laboratory testing.

The restaurant inspection resulted in the identification of a number of high-risk foods containing undercooked egg. Details of the restaurant's egg supplier were obtained and a site visit of the egg supplier was arranged.

None of the food served at the function remained. An environmental swab from the KitchenAid™ was collected in addition to representative samples of menu-items containing raw or lightly cooked egg. Food samples collected included homemade ice-cream, chocolate mousse, eggs, and aioli. These samples were submitted to the MDU. A second restaurant inspection was conducted on 29 May to obtain further information on food preparation methods.

4.2.2 Egg supplier inspection

The Principal Veterinary Officer, together with the local District Veterinary Officer, conducted an inspection of the egg farm on 25 May. An Egg Standards Inspection Report was completed, which included assessment of egg processing and inspection methods, as well as physical inspection of the free range chicken sheds and flock health. A swab of chicken faecal matter was

collected from the floor of both chicken sheds in order to test the flock for *S.* Typhimurium. These samples were submitted to the diagnostic laboratory at AgriBio.

4.2.3 Laboratory investigation

Faecal samples obtained from function attendees who had become ill, and had not previously had a sample taken, were submitted to MDU for culture and MLVA testing. The MLVA pattern was used for active-case finding by comparing the MLVA pattern of the outbreak strain to that of *S.* Typhimurium cases notified to the DHHS in the month prior to the outbreak. Closely related MLVA strains are as those with the number of tandem repeats differing by one or two numbers at one of the second to the fourth loci (23). Any cases with an identical or closely related MLVA strain were contacted to obtain a history of foods consumed in the three days prior to onset of illness in order to assess if the case had attended the restaurant where the outbreak had occurred.

4.3 Ethics approval

This study was conducted as an outbreak investigation under the *Victorian Public Health and Wellbeing Act 2008*. This study was also covered by a waiver of consent under ANU HREC protocol 2017/909.

5. Results

5.1 Epidemiological investigation

Thirty-nine of the 53 function attendees were interviewed, a response rate of 73.6%. One guest was not able to be contacted and a dining group of 13 people declined to participate in interviews. Nineteen attendees reported becoming ill after eating at the restaurant, an attack rate of 48.7%. Of the 19 attendees, there were six probable and 13 confirmed cases.

Among the 19 cases, 10 attended the 11:00 sitting and nine attended the 14:00 sitting. Of these, nine were male (47.4%), and 10 were female (52.6%). The median age of function attendee cases was 45 years, with a range of 10 to 81 years. The median age of all function attendees was 48 years, with a range of 8-81 years.

The majority of function attendees were interviewed within two weeks of the function (53.8%). All interviews were completed within 19 days of the function. Among the cases, interviews were completed a median of 10 days after the function. Of the entire cohort, interviews were completed a median of 12 days following the event.

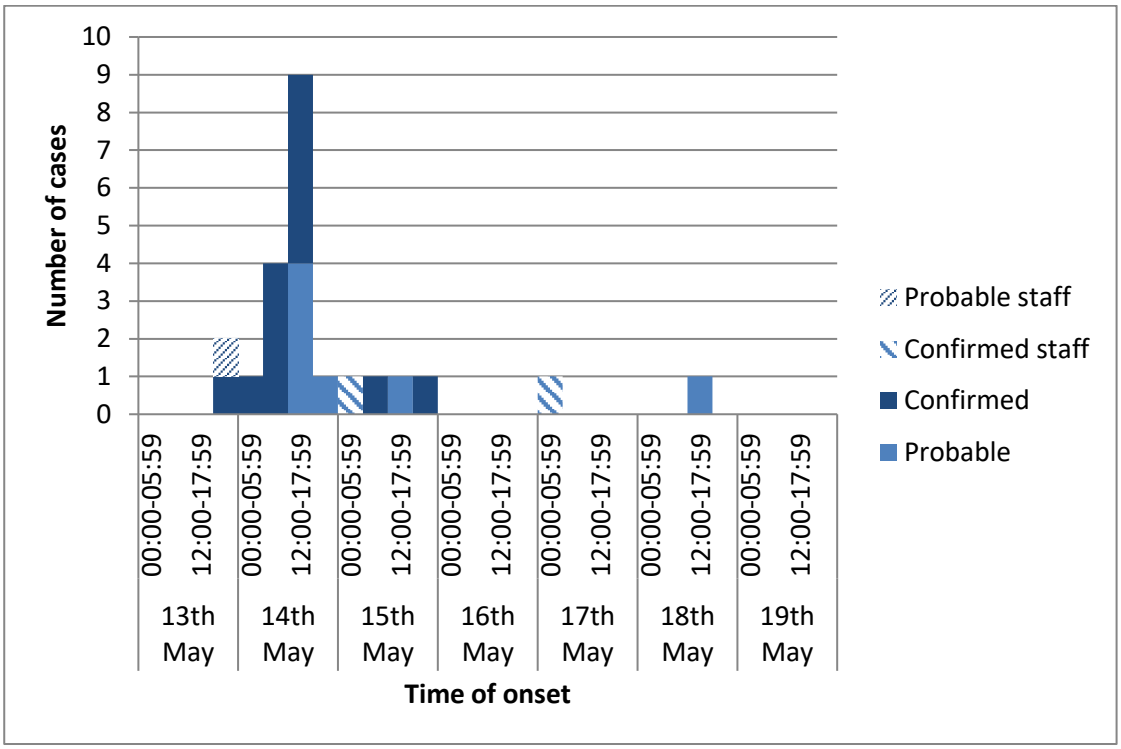
Of the 19 cases, 15 sought medical care (78.9%), four attended an emergency department (21.1%) and two people required admission to hospital (10.5%). The majority of cases had symptoms including fever (78.9%), nausea (73.7%) and abdominal pain (89.5%) (Table 1). Less than half of the cases (42.1%) experienced vomiting. All cases had diarrhoea, of which one had bloody diarrhoea.

Table 1: Symptoms of cases who attended a Mother's Day function on 13 May 2018 in Victoria

Symptom	Number	Percent of all cases (n=19)
Fever	15	78.9%
Nausea	14	73.7%
Vomiting	8	42.1%
Abdominal pain	17	89.5%
Diarrhoea	19	100.0%
Watery diarrhoea	18	94.7%
Bloody diarrhoea	1	5.3%
Headache	12	63.2%

The median incubation period was 27.5 hours after attending the function with a range of nine to 53.5 hours. The time of onset was not reported by three cases, therefore an incubation period could not be calculated for these cases. The median duration of illness was eight days (range four to 14 days). Illness duration was not reported for seven cases. The epidemic curve was consistent with a point source outbreak (Figure 1). The number of cases peaked between midday and 18:00 on 14 May.

Figure 1. Epidemic curve displaying number of ill function attendees and staff by onset date and time[1]



Among the menu items, the attack rate of the chocolate mousse (80.0%) was highest (Table 1). Univariate analysis of each menu food item found that the risk ratio of illness among those who consumed chocolate mousse served with coffee anglaise was highest (RR 5.1, 95% CI: 1.8–14.6).

5.1.1 Interviews of ill staff

After working at the restaurant on the day of the Mother's Day function and/or being directly involved in food preparation the day prior to the function, three members of staff including the apprentice chef and two food handlers became ill. All ill staff had symptoms consistent with salmonellosis including watery diarrhoea and abdominal pain. Two of these staff members had laboratory-confirmed salmonellosis.

The structured menu-based questionnaire was not administered to these staff, however a three day food history questionnaire was completed. These staff recalled tasting various food items including hot chips with aioli, chocolate mousse, and coffee anglaise while at work, however they were not able to recall specific details. These staff may have consumed various other items

[1] An onset of 12:00 on the date of onset is assumed for three cases where a time of onset was not recorded

in the restaurant. All ill staff were advised not to attend work until their symptoms resolved. Staff were not included in the epidemiological analysis.

Table 1: Univariate analysis of risk ratio (RR) for individual menu items. Values meeting statistical significance of $p<0.05$ are bolded.

Food item	Exposed			Not exposed			RR (95% CI)	Exact logistic regression OR (95% CI)	*p* value
	Ill	Not ill	Attack Rate	Ill	Not ill	Attack rate			
Salami	16	15	51.6%	0	1	0	-	0.9 (0.0–∞)	1.000
Mustard	8	10	44.4%	8	7	53.3%	0.8 (0.4–1.7)	-	0.611
Cornichon	12	13	48.0%	3	4	42.9%	1.1 (0.4–2.9)	-	0.810
Jamon (Spanish ham)	15	15	50.0%	0	1	0	-	0.9 (0.0–∞)	1.000
Croquettes	19	18	51.4%	0	2	0	-	2.4 (0.2–∞)	0.487
Aioli with croquettes	13	13	21.4%	2	5	28.6%	1.8 (0.5–6.0)	-	0.312
Salmon	14	15	48.3%	4	4	50.0%	1.0 (0.4–2.1)	-	0.931
Oysters	10	14	41.7%	9	6	60.0%	0.7 (0.4–1.3)	-	0.265
Beef	9	6	60.0%	10	14	41.7%	1.4 (0.8–2.7)	-	0.265
Duck	3	6	33.3%	16	14	53.3%	0.6 (0.2–1.7)	-	0.292
Barramundi	9	10	47.4%	10	10	50.0%	1.0 (0.5–1.8)	-	0.870
Fries	16	14	53.3%	1	3	25.0%	2.1 (0.4–12.0)	-	0.287
Aioli with fries	7	6	53.9%	9	6	60.0%	0.9 (0.5–1.7)	-	0.743

Broccolini	12	17	41.4%	2	2	50.0%	0.8 (0.3–2.4)	-	0.744
Radish and shallot salad	15	12	55.6%	4	6	40.0%	1.4 (0.6–3.2)	-	0.401
Quince tart	5	5	55.0%	14	15	48.3%	1.0 (0.5–2.1)	-	0.925
Crème brulee	6	8	42.9%	11	12	47.8%	0.9 (0.4–1.9)	-	0.769
Chocolate mousse	**16**	**4**	**80.0%**	**3**	**16**	**15.8%**	**5.1 (1.8–14.6)**	**-**	**<0.001**
Cheese platter	0	4	0.0%	19	16	54.3%	-	0.2 (0.0–1.5)	0.106
Aioli overall	14	13	51.9%	5	5	50.0%	1.0 (0.5–2.1)	-	0.920
Chicken	0	1	-	2	1	66.7%	-	1.0 (0–39)	1.000
Fish and chips	2	1	66.7%	0	1	0	-	1.0 (0.0–∞)	1.000
Icecream	2	2	50.0%	0	0	0	-	-	-
Cake	0	0	-	19	18	51.4%	-	-	-

5.2 Environmental investigation

5.2.1 Restaurant inspection and food preparation interrogation

The restaurant inspection found no obvious issues with hygiene or food storage. On the day of the function, a set menu was provided with a number of shared entrée, main, side dishes, and desserts served. The menu included several foods that contained raw or lightly cooked egg: aioli, chocolate mousse served with coffee anglaise, and home-made icecream. Children had the option of ordering from a separate menu with a limited number of items.

The aioli and the chocolate mousse were prepared the evening prior to the function. The aioli contained raw egg, while the chocolate mousse contained egg yolk, which was lightly cooked over a water bath prior to storage in a cool room. Preparation of the chocolate mousse involved egg separation, which was performed by hand using the shell-shell method. Coffee anglaise accompanied the chocolate mousse and contained egg yolks, which had been heated to approximately 80°C in a Thermomix®. The homemade ice-cream also contained egg yolks which were cooked to approximately 80°C prior to being frozen.

Staff interviews revealed that the eggs used for the above dishes had been delivered on 09 May and that the person delivering the eggs advised that they were softer than normal. The delivery person advised that this may have occurred as the farm had been in the process of removing old chickens and introducing new chickens. Both the head and assistant chef prepared the chocolate mousse and tasted it, reporting that the mix was softer than normal, potentially indicating that the eggs used were more runny than usual.

5.2.2 Egg supplier inspection

The inspection of the egg farm supplying the restaurant found no major issues. The farm operated a free-range system with chickens appearing to be in good health. The owner of the farm was unsure about the use of vaccines for *Salmonella* at the breeder from which he purchases the chickens. The condition of the chickens was checked on a daily basis and drinking water was free from contamination. Eggs are collected from the paddocks by hand and any soiled or cracked eggs being disposed of at the time of collection. The owner reported that egg washing is not performed as minimal numbers of soiled eggs are produced; small amounts of dirt on the surface of eggs are removed with sandpaper or a dry cloth. No records of cracked or discarded eggs were kept.

Eggs were visually inspected for cracks in the egg-processing shed. No handwashing facility was present in the egg sorting room; staff currently use the facilities at an adjacent restaurant. The Principal Veterinary Officer recommended that the owner inspect eggs for cracks using the candling method, as visual inspection may miss hairline cracks on the surface of the eggs. It was also recommended that handwashing facilities are installed in the sorting room as soon as possible and that records of cracked and soiled eggs should are kept at egg collection points. The egg supplier was advised to obtain chickens from a breeder who vaccinates their flock for *Salmonella.*

5.2.3 Laboratory results

Samples of the homemade ice-cream, chocolate mousse, eggs and aioli, and the environmental swab from the KitchenAid™ obtained from the restaurant were negative for *Salmonella.* The two environmental samples obtained from the egg farm were negative for *Salmonella.* Among the function attendees there were 13 laboratory confirmed cases of *S.* Typhimurium. Among the staff there were two confirmed cases of *S.* Typhimurium. All confirmed cases had the same MLVA pattern of 03-15-11-10-523.

In the 30-day period prior to the outbreak, six cases with a similar or identical MLVA pattern had been notified to the DHHS. All of these cases were interviewed to obtain information on foods eaten in the three days prior to becoming ill. However, none of these cases reported dining at the restaurant involved in this outbreak and they were therefore excluded from the investigation.

6. Discussion

6.1 Restaurant inspection and epidemiological investigation

The epidemiological findings of the cohort study strongly supported the hypothesis that a food containing undercooked egg was the vehicle for salmonellosis. Eggs are frequently associated with outbreaks caused by *S.* Typhimurium and the consumption of raw or lightly cooked egg is a common factor in many previous outbreaks (15, 25-30). As indicated in this investigation, desserts containing egg that have not been heated adequately can act as a vehicle for the transmission of *Salmonella* (20).

Despite a lack of microbiological evidence from food sampling in this investigation, univariate analysis strongly suggests that the chocolate mousse was the most likely vehicle for *Salmonella.*

In the cohort, those who consumed the chocolate mousse were five times (RR 5.1, 95% CI 1.8–14.6) more likely to become ill, compared to those who did not consume the mousse.

Negative culture results for the food samples tested in this investigation do not eliminate the chocolate mousse or the eggs as the underlying source of *Salmonella* in this outbreak. By the time of the restaurant inspection, all food served at the function associated with the outbreak had been discarded. Furthermore, *Salmonella* can be difficult to isolate from affected eggs (2). A study conducted from 2001–2009 found that *Salmonella* was only detected in eggs in 39% of outbreaks investigated where eggs were implicated (15). Even in experimentally infected chickens with confirmed colonization of reproductive tissue, *S.* Typhimurium was unable to be cultured from the internal contents of eggs (31).

Food preparation techniques are an important aspect of preventing food-borne disease. The cooking process for the chocolate mousse was one of the main risks identified at the restaurant in relation to this outbreak. The chocolate mousse contained egg, which had been lightly cooked over a water bath of unspecified temperature. To minimise the risk of *Salmonella* transmission, egg should be adequately cooked to more than 75°C (19). The shell-shell separation method of eggs used for the chocolate mousse may have caused the egg contents to be contaminated by the eggshell. A sanitised egg separator is the recommended method for egg separation (14, 19). Although the coffee anglaise was served with the chocolate mousse, it contained egg that had been heated to 80°C and was therefore an unlikely vehicle for *Salmonella.*

The restaurant inspection also identified the use of raw egg in aioli, another high-risk food for *Salmonella*. The aioli was not implicated as a food vehicle in this outbreak. The aioli was served with both the croquettes and the fries and had a risk ratio of 1.0 (95% CI 0.5–2.1). This indicates that there was no increase in the risk of becoming ill in those who consumed the aioli, compared to those who did not. It is likely that only a small number of eggs within the batch used to prepare food for the function were contaminated with *Salmonella.* It is possible that by chance, the eggs used to prepare the aioli were not contaminated.

To minimise the risk of future outbreaks of salmonellosis, recommendations for the restaurant include the use of safer alternatives to raw or lightly cooked egg including pasteurised egg products, liquid, frozen or dried processed eggs, or sugared eggs for use in desserts (19). As some restaurants prefer not to use these alternative products, restaurant staff were also educated on the safe preparation of egg-based foods.

The requirement for a second restaurant visit highlighted the need for standardised data collection when conducting restaurant inspections as part of gastroenteritis outbreak investigations. It also indicated the need for regular training of EHOs, who may only sporadically be involved in outbreak investigation. Since this investigation the *Victorian Guidelines for the investigation of gastroenteritis* (32) have been revised. These revisions may contribute to the improved conduct of future investigations.

6.2 Investigation of the egg supplier

S. Typhimurium is thought to be endemic in Australian layers, however data on the prevalence of *Salmonella* contaminated eggs, and the prevalence on Australian egg farms is limited (15, 33). The negative results for environmental samples obtained from the egg farm do not eliminate eggs as the underlying source of *Salmonella* in this outbreak. Faecal shedding of *Salmonella* may not accurately indicate the presence in the caecum of affected chickens (34). Furthermore, there is no correlation between the level of *Salmonella* in faeces and the recovery of *Salmonella* from the surface of the egg shells in experimentally infected chickens (31). Chickens colonised by *S.* Typhimurium are likely to shed the organism intermittently, with the amount shed varying with the stress levels of the individual, the season and the stocking density (9, 31). As *Salmonella* is shed intermittently the organism is difficult to isolate from infected flocks and successful isolation therefore depends on the number and type of samples collected (9). As only a single sample was collected from each chicken shed in this investigation, the negative results may be a result of intermittent shedding.

The results of restaurant staff interviews suggest that the egg supplier's chicken flock had recently been changed over. The change-over process is likely to act as a source of stress for the flock, potentially precipitating the shedding of *Salmonella* (31). As the eggs used to prepare the food for the function were delivered four days prior to the restaurant function and more than a week before the egg farm was inspected, the samples taken may not have originated from the same flock that had laid the eggs implicated in this outbreak.

To reduce the number of outbreaks associated with the consumption of eggs, ongoing education of consumers and food-preparation staff and businesses is needed, however measures to minimise bacterial contamination of eggs at the farm level are also required (21). Any cracks and hairline fractures of the eggshell may provide a route for *Salmonella* to enter the internal egg contents (20). It is therefore recommended that the farm implement egg-candling practices to reduce the chance of missing small cracks in their egg supplies. Vaccination of chickens with a mutant live attenuated vaccine provides some protection against intestinal infection and can

decrease the risk of egg contamination with *Salmonella* by reducing the possibility of shedding (9, 35). The farm inspected during this investigation was unaware of the availability of the *Salmonella* vaccine, and will obtain information on the vaccination status of the farm from which chicks are purchased.

6.3 Limitations

Limitations of this cohort study included a lack of microbiological evidence to support the epidemiological findings, and potential recall bias for some interviews. As the majority of function attendees were interviewed within two weeks of the outbreak, recall bias is likely to be minimal for the cohort study. However, some interviews were completed up to 19 days after the outbreak occurred, due to difficulties contacting several function attendees. Recall bias may have been significant for a small number of participants who were interviewed more than two weeks after attending the function. Recall bias may have caused differential misclassification of exposure, as function attendees without illness may have been less likely to recall menu items consumed, compared to attendees who experienced illness. This may have biased the results away from the null. This was somewhat unavoidable as CDPC was not notified of the outbreak until eight days after the restaurant function.

The use of a structured outbreak questionnaire minimised the occurrence of interviewer bias. The outbreak investigation team included several highly experienced PHOs who assisted with conducting interviews of function attendees. Due to their experience, most of the PHOs were likely aware that a food item containing raw or lightly cooked egg was the source of the outbreak from the outset of the investigation. Providing those who conducted interviews with a structured set of interview questions ensured that PHOs asked about all individual menu items consumed, regardless of their suspicions regarding the source of the outbreak.

There was a lack of microbiological evidence to support the chocolate mousse or the eggs as the causative agent of the outbreak, as all of the food items served at the function had either been discarded or consumed by the time the restaurant inspection was conducted. Furthermore, as it is possible that the swabs collected at the egg farm were collected from a different flock of chickens to those that laid the implicated eggs, it is not possible to definitively confirm the source of the *Salmonella* in this outbreak.

7. Conclusions

This outbreak was one of the many *Salmonella* outbreaks associated with egg-based foods each year. The environmental investigation highlighted several risky food preparation practices in place at the restaurant, and enabled restaurant staff to be educated on the risks associated with the preparation of egg-based dishes. Restaurant staff were also provided with recommendations on changes in practices to minimise the risk of future outbreaks.

The site visit to the egg farm enabled some issues in the production chain to be identified, and provided an opportunity for education of the producer regarding ways in which the risk of *Salmonella* contamination of eggs can be minimised. Ongoing education of Victorian food businesses, food preparation staff, egg producers and consumers is essential to minimise the number of egg-associated *Salmonella* outbreaks in the future.

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Appendix 1. Presentation at National Centre for Epidemiology and Population Health Lunchtime Seminar. Wednesday 27 February, 2019

Salmonellosis at a Mother's day lunch in regional Victoria

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And

The Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL)

Background - Salmonellosis

- Common foodborne pathogen causing gastroenteritis
- *Salmonella* Typhimurium is the most commonly notified serotype
- Often associated with consuming undercooked eggs
- In Victoria, all Typhimurium isolates typed using MLVA (Multiple Variable Analysis of Tandem Repeats)

Notification of an outbreak

Mother's day function 13th May 2018

Several patrons with gastroenteritis complained to restaurant

Restaurant notified local council

Council notified DHHS (18th May 2018)

Lunch menu

- Several high risk items for Salmonella identified
- Arranged inspection of egg supplier farm for the following week

Salami	Mustard	Cornichon	Jamon (Spanish ham)
Croquettes	Salmon	Oysters	Beef
Duck	Bernaumdi	Fries	Broccolini
Radish and shallot salad	Guinea fan	Crème brulee	Chocolate mousse
Cheese platter	Aioli	Chicken	Fish and chips
	Icecream	Cake	

Restaurant inspection + staff interviews

- Review of food processes
- Environmental health officers (EHOs) obtained 3 day food history from unwell staff
- Representative samples of items from function menu collected
- Samples submitted to MDU PHL

Environmental investigation findings

- Aioli contained raw egg
- Chocolate mousse contained egg cooked over a water bath of unknown temperature
- Staff interview revealed that egg supplier had been in the process of changing over chicken flock

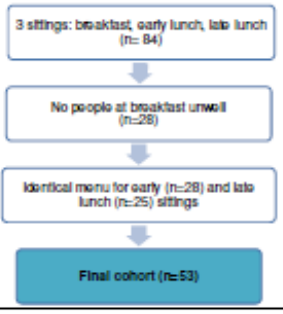
Environmental investigation: Egg farm inspection

- Small free range chicken farm
- Chickens in good health
- Owner unaware about *Salmonella* vaccination status
- Visual inspection of eggs only

Environmental investigation: Egg farm inspection

- 'Drag swab' of chicken faeces from floor of chicken shed collected

Epidemiological investigation



Epidemiological investigation

- Retrospective cohort study
- Structured outbreak questionnaire designed based on set lunch menu
  - If unwell, asked to provide faecal sample
- 74% (n = 39/53) response rate
  - Group of 13 declined to participate

Cohort study: Case definitions

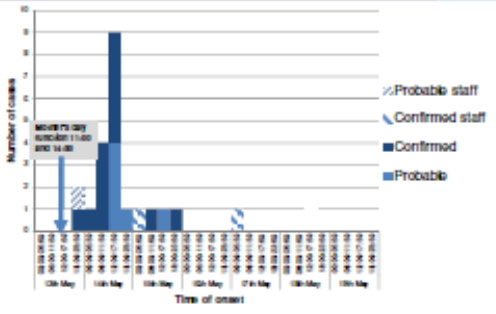
Probable case: A person who ate at the restaurant on the 13th of May, 2018 (or was involved in food preparation for the function) who subsequently developed **gastroenteritis within 72 hours of the function**

Confirmed case: A person who ate at the restaurant on the 13th of May, 2018 (or was involved in food preparation for the function) who subsequently developed gastroenteritis and had a **faecal sample that tested positive for S. Typhimurium MLVA 03-15-11-10-523**

Cohort study results

- 9 males (47%), 10 females (53%) unwell
  - Median age unwell function attendees 45 years (10-81 years)
- Even proportion (36%) of attendees unwell between early and late lunch sittings
- Median incubation period of 27 hours (range 9 to 53 hours)
- 15 cases (79%) saw a GP, 4 (21%) attended emergency, 2 (10%) admitted to hospital

Epi curve



Univariate analysis

	Ill	Not ill	Attack Rate	Ill	Not ill	Attack rate		

Attack rate of chocolate mousse = 80% (RR 5.1, 95% CI 1.8-14.6, $p<0.001$)

Laboratory results

- Food samples from restaurant all negative
- Environmental samples from egg farm negative
- 13 function attendees and 2 staff had laboratory confirmed Salmonellosis (all with MLVA pattern 03-15-11-10-523)

Investigation outcomes

- Chocolate mousse was the likely food vehicle for *Salmonella*
- Restaurant staff educated about appropriate cooking practices for dishes containing lightly cooked egg
  - use of pasteurised egg products

Investigation outcomes

- Egg supplier educated advised to implement candling to check eggs for cracks
- Sourcing chicks from a flock vaccinated for *Salmonella*

Thank you

- **Communicable Diseases Prevention and Control team at the Victorian Department of Health and Human Services:** liaison with EHOs and the restaurant, assisted with case interviews
- **Marion Easton** – DHHS field supervisor
- **Emma Field** – academic supervisor
- **Zoe Cutcher** – Victorian OzFoodNet Epidemiologist
- **Dr. Joni Segel** – Principal Veterinary Officer
- **Interview participants**

Chapter VI: Recruitment of Case-controls for a Multi-Jurisdictional Outbreak of Hepatitis A

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1. Preface

OzFoodNet conducts surveillance of foodborne disease in Australia. In early April 2018, OzFoodNet instigated a multi-jurisdictional outbreak investigation (MJOI) in response to seven cases of locally acquired hepatitis A virus (HAV). All cases were genotype IB, and six of seven cases reported the consumption of frozen pomegranate. Commencing on 13 April 2018, a national case control study was led by Health Protection New South Wales (NSW).

Dr Zoe Cutcher, an OzFoodNet Epidemiologist based at the DHHS, requested my assistance with interviewing case controls as part of the MJOI. I conducted interviews from late April to mid-May 2018. As I was not involved in data analysis or the synthesis of the outbreak report, I have not reported the study's findings or methodology in detail. This chapter describes my experience of recruiting case controls as part of this investigation and provides a synopsis of the case-control study findings as reported by OzFoodNet.

1.1 My role

I was responsible for recruiting Victorian controls as part of the MJOI, with the aim of identifying and interviewing two controls for each case.

- I liaised with Victorian OzFoodNet Epidemiologists in order to obtain and clarify the details of the study protocol and the contact details for potential controls
- I contacted controls, assessed their eligibility for inclusion in the case-control study and conducted case-control interviews as per the study protocol
- Once the control questionnaire was completed for the HAV MJOI, I also administered standard salmonellosis, campylobacteriosis or cryptosporidiosis case questionnaires to assess risk factors and obtain three day food histories, to be utilised if the person was subsequently included in a gastroenteritis outbreak investigation
- I entered standard questionnaire results into the Public Health Event Surveillance System (PHESS) and liaised with OzFoodNet Epidemiologists once case-control interviews were complete

1.2 Lessons learnt

This investigation demonstrated the resource-intensive nature of case-control studies. The volume of calls and interviews required to recruit controls enabled me to refine and enhance

my skills in conducting questionnaires. The interview process also highlighted the potential for recall bias within case-control studies.

1.3 Acknowledgements

I acknowledge the following people for their assistance with this project:

- Dr Zoe Cutcher and Joy Gregory, Victorian OzFoodNet Epidemiologists, who provided me with the contact details for controls, and additional controls when I had exhausted the original possibilities
- Ana-Lena Arnold, Epidemiologist at the DHHS, for sharing her experiences recruiting controls for a previous foodborne Hepatitis A MJOI, and for encouraging me to include this activity in my bound volume
- Kaye Sturge, Senior Public Health Officer, for assisting me with the logistics of completing interviews and providing advice on how to conduct them efficiently and accurately
- All participants who completed questionnaires as part of this investigation

2. Background

HAV is transmitted by the faecal-oral route and can be spread by direct contact or through the consumption of contaminated food (1-3). Infection causes fever, inappetance, nausea, fatigue, abdominal pain, diarrhoea and jaundice which may progress to liver failure (1, 2, 4, 5). While adults usually develop symptomatic infection, infected children may remain asymptomatic (5). HAV has an average incubation period of 30 days, ranging from 15-50 days (1, 2). Consequently, as an infected person starts to shed the virus in faeces prior to the development of symptoms, the virus can be transmitted while asymptomatic (1, 2).

Areas including South East Asia and Africa are endemic for HAV (6). Countries with a low incidence of HAV, including Australia, often import frozen food products from countries endemic for HAV, creating a potential route for foodborne transmission. Over the past five years there have been several foodborne outbreaks of HAV worldwide, associated with the consumption of semi-dried tomatoes, frozen pomegranate and frozen berries (2, 4-11). In Australia in 2015, a MJOI of HAV was associated with the consumption of imported frozen mixed berries (12).

HAV is a non-enveloped RNA virus able to persist for long periods in the environment (1). When present on frozen fruit, HAV can remain infectious for months (13). As frozen fruit products pass through multiple processing stages including sorting, washing, draining and packing, there are several points at which HAV contamination may occur (9).

There are six HAV genotypes, with I to III causing infection in humans (11). The genotypes are subdivided into A and B (11). HAV is difficult to grow in cell culture and is rarely done. Therefore, molecular testing including PCR is used to detect HAV and genomic sequencing uses the PCR product to subtype the virus (1).

Three cases of HAV, genotype IB, were identified in NSW in late March 2018. This genotype had not been previously detected in Australia. The cases had no history of overseas travel, and illness appeared to be associated with the consumption of imported frozen pomegranate arils (14). Four additional cases of locally acquired HAV were identified across four jurisdictions. Of a total of seven locally acquired HAV cases, six had consumed frozen pomegranate. Food Standards Australia and New Zealand subsequently implemented a national recall of frozen pomegranate on 7 April 2018, and OzFoodNet initiated a MJOI on 10 April 2018.

3. Methods

3.1 Study design

A prospective, frequency matched case-control study was conducted to obtain epidemiological evidence for the association of HAV with the consumption of frozen pomegranate (14). Each jurisdiction was responsible for interviewing cases and controls residing within their state.

For each case of HAV, two controls were recruited. Controls were frequency-matched to cases by age group. Controls were also matched by local government area (LGA) and where possible, sex. At the DHHS, controls were identified from other notifiable enteric disease cases stored in the notifiable disease database, PHESS. Controls were initially selected from notifications of salmonellosis, followed by campylobacteriosis, and lastly, other cases of notifiable diseases such as cryptosporidiosis.

The case-control questionnaire commenced with questions to assess eligibility to be a control and obtain information on demographics. Eligible controls were then asked questions that assessed their exposure to a range of fresh and frozen food items in the five weeks prior the onset of their notifiable illness. This period was chosen to coincide with the incubation period for a HAV case. The participants were also asked about their awareness of the recent recall of the frozen pomegranate product.

Whole genome sequencing often takes some time to be completed. This means that there were several Victorian locally acquired cases of HAV suspected to be part of the MJOI, pending sequencing results. To ensure that case-controls were interviewed as soon as possible, the control recruitment process was therefore initiated prior to confirmation of cases as HAV genotype IB.

3.1.1 Case definition

As per the study protocol, probable cases had to satisfy the following three conditions (14):

- a person infected with HAV, with sequencing results pending or unavailable
- with an onset of illness from 1 January, 2018
- had spent some of their incubation period (15-50 days prior to onset of illness) in Australia

Confirmed cases were those infected with HAV, genotype IB, with an onset from 1 January, 2018, and spent some of their acquisition period (15-50 days before onset of illness) in Australia (14).

3.1.2 Selection of controls

Controls had to satisfy the following three conditions:

- notified to the DHHS and with a specimen collection date in the two weeks before the onset of the corresponding HAV case
- same age group as the case (e.g. 0 to 14 years)
- with a residential address in the same LGA as the HAV case

When a control could not be identified from the same LGA, they were selected from neighbouring LGAs. Controls with a specimen collection date closest to the onset date of the corresponding case were prioritised.

3.1.3 Eligibility of controls

Controls who had lived in a country with a high incidence of HAV for more than a year in the first five years of life were excluded from the study. In these countries, HAV is often contracted as a child, therefore the majority of adults have HAV IgG antibodies (6).

Controls who met any of the following conditions were excluded (14):

- Previous infection with HAV or symptoms (e.g. jaundice) that may indicate previous HAV infection
- Previous vaccination for HAV
- Previous treatment with normal human Immunoglobulin in the two months prior to the onset of diarrhoea
- Salmonellosis or campylobacteriosis cases currently included in an outbreak investigation
- People who are not contactable by mobile or landline telephone following two attempts during business hours and two attempts after hours
- People who do not speak English or who cannot answer questions coherently
- People with a history of overseas travel in the two months prior to the onset of diarrhoea
- People with close contact with someone known or thought to have HAV in the two months prior to the onset of diarrhoea

Once completed, questionnaires were provided to an information officer within the DHHS, to be entered into SharePoint.

4. Results

4.1 Recruitment of case-controls in Victoria

Over a two and a half week period, I made 76 phone calls as part of the study. This does not include additional phone calls which were sometimes required to obtain or confirm the control's contact information, or to determine the referring doctor, if these details had been not been provided on the notification form.

For four cases of HAV I successfully recruited eight controls over a two to three week period. As part of this process, I excluded 21 potential controls from inclusion in the study. This included:

- Seven controls excluded due to previous HAV vaccination
- Two controls excluded due to previous HAV infection or symptoms potentially indicating previous HAV infection
- Five people excluded due to travel overseas in the two months prior to developing diarrhoea
- Five people who were unable to be contacted
- One person excluded due to birth in a country that is endemic for HAV
- One person excluded as they were unable to coherently complete the questionnaire

4.2 Summary of the MJOI (14, 15)

Australia wide, a total of 30 cases met the confirmed case definition (14). There were 15 (50%) cases in NSW, six (20%) in Victoria, three (10%) in Western Australia, two (7%) each in South Australia and the Northern Territory, and one (33%) each in Queensland and the Australian Capital Territory.

Primary outbreak cases are those thought to have contracted HAV from the consumption of imported frozen pomegranate. There were 27 primary outbreak cases (90%), of which 18 (67%) recalled consuming frozen pomegranate during their incubation period for HAV. Two cases recalled consuming frozen pomegranate in a salad purchased from a café, and one case was too unwell for interview, however frozen pomegranate was found in the case's freezer

indicating possible consumption. Five cases (19%) could not recall consuming any pomegranate during their incubation period.

Only one case, from NSW, reported a history of travel, to the USA, during their incubation period for HAV. There were three secondary cases, all in NSW, with epidemiological links to confirmed cases. These secondary cases were likely acquired through sexual contact.

Twenty five cases (83%) required hospitalisation. One case residing in South Australia died, however it was unclear if this was as a direct result of HAV infection.

4.3 Case-control study

Thirteen cases of HAV genotype IB and twenty-one controls were included in the case control study. Victoria contributed seven controls for six cases. Univariate analysis for the association of infection with HAV genotype IB and the consumption of food items was conducted. Analysis identified an association between HAV infection and the consumption of several frozen fruits, with the strongest association identified for frozen pomegranate (Odds ratio 45.0, 95% CI 3.8-2065.4, p<0.001)(14).

4.4 Environmental investigation

The frozen pomegranate implicated as the causative agent of this outbreak was imported from Egypt and had been distributed throughout Australia. A trace back investigation found that the pomegranate was most likely contaminated with HAV before importation into Australia. The Egyptian government were therefore responsible for investigation into the possible cause of the contamination.

Nucleic acid testing of frozen pomegranate samples found one packet that was positive for HAV however the virus was not able to be typed. Any remaining frozen pomegranate was collected from the houses of HAV cases, as well as from retail and distribution centres.

4.5 Control measures

Subsequent to a national recall of the brand of frozen pomegranate implicated in this outbreak, individual jurisdictions published media releases regarding the outbreak's association with frozen pomegranate. No further cases of HAV genotype IB were identified. Following the MJOI, the Australian Government implemented inspection and testing of all imports from the manufacturer of the pomegranate associated with the outbreak.

5. Discussion

This investigation taught me that recruiting controls can be a time-consuming and challenging process which requires persistence. At times, organising the most convenient time for interview was difficult and required multiple voicemails or after-hours phone calls over several days. Due to the number of controls that were excluded from the study and the time spent undertaking this process, it is likely that a degree of recall bias existed for some participants. As some controls were contacted many weeks after their potential exposure period, they may have been less likely than HAV cases to accurately recall the foods that they consumed. This may have resulted in differential misclassification of exposure to the food item responsible for the outbreak between cases and controls, and therefore biased the study results away from the null.

My involvement in this investigation has enabled me to become confident in conducting case-control questionnaires. The process of control recruitment required me to clearly explain the reason for calling and to clarify the rationale for the case-control questionnaire. I also learnt the importance of adhering to the written wording of the questions in the structured case control questionnaire and ensuring that a clear “yes”, “no” or “don’t know” answer was obtained for each question.

At times throughout the recruitment process it was difficult to ensure the exclusion criteria were accurately adhered to. Many controls were unable to remember if they had been vaccinated for HAV in the past, or how many vaccinations they had received. I often had to ask several follow up questions to determine the likelihood of previous vaccination. If there was any possibility of previous vaccination, I deemed the control ineligible and they were excluded from the study. Adhering to the study criteria was important as previous HAV vaccination would reduce the risk of contracting the infection, causing differential misclassification of exposure. Inclusion of previously vaccinated controls in the study may have biased analysis results towards the null.

As part of the case-control questionnaire, all participants were asked if they were aware of the recent recall of frozen pomegranate, as this was the implicated food vehicle for the HAV outbreak. Interestingly, the majority were unaware of the recall. In future, consideration should be given to expanding the ways in which food recall information is disseminated.

6. Conclusion

My involvement in this case control study enabled me to understand some of the complexities in the study design and recruitment of controls in case-control studies. My experience conducting interviews also demonstrated the potential sources of bias in case-control studies.

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Chapter VII: Teaching

1. Introduction

MAE scholars are required to be involved in two teaching activities to meet a teaching competency. These include designing and presenting a lesson to the first year MAE cohort, in addition to teaching a lesson from the field (LFF) to a group of our peers. My LFF was conducted within a group of five other MAE scholars.

1.1 Lesson from the field

Within the first few weeks of my field placement, I was asked to analyse data from the Victorian Seasonal Influenza Survey. This survey was designed by the media team at the DHHS to ascertain the level of knowledge of influenza among the general population of Victoria. The aim of the survey was to understand how the vaccination rates for influenza could be improved over the coming year. The findings of the survey were planned to be used to inform the media team's upcoming flu campaign.

I was very new to the field of epidemiology and data analysis at the time I was given this project, however the analysis was made more challenging due to several issues identified in the design of the survey. For example, there didn't appear to be a clear hypothesis, and some of the questions had overlapping answer options. I later identified this scenario as a good opportunity for an LFF on the topic of survey design.

I had approval to provide my LFF group with a copy of the survey for the lesson. I designed the LFF to encourage critical evaluation of the survey questions. The learning objectives of the LFF were to understand the steps and potential pit-falls associated with questionnaire design, and to demonstrate the importance of the steps of survey design and careful wording of questions. The LFF also provided a brief introduction to some methods that are available for the analysis of multiple-response questions in STATA.

1.2 Teaching session for the first year MAE scholars

I worked within a group of three MAE scholars to design and present a 30-minute session on data visualisation. The learning objectives were to gain an understanding of the importance of data visualisation and to introduce the variety of methods available. To make the lesson engaging, we also included examples of data visualisation for the students to evaluate and presented a demonstration using Power BI, a business analytics data visualisation software.

The sessions were evaluated using a brief questionnaire completed on the day. First year scholars were asked to rate the clarity of the learning objectives, the level of engagement of the presenters, the pace of the session and the usefulness of the content. Overall, students found our session useful and engaging with clear objectives.

In addition to the above, I also participated in teaching an existing outbreak investigation case study to the first-year scholars. This involved leading a small group discussion together with another student within my cohort, as part of a sixty minute session.

1.3 Lessons learnt

My involvement in the above activities has confirmed my enjoyment teaching and collaborative discussions. The teaching session for the first years also further developed my public speaking skills.

The experience of teaching the first year MAE scholars taught me the importance of writing presentations that are tailored to the audience. In future when teaching a postgraduate session, I would further investigate the level of prior knowledge of the students. The 2019 MAE cohort is particularly experienced and knowledgeable, therefore our session could have gone into further detail. Next time I am involved in teaching as part of a course, I would also obtain additional information on the content that had already been delivered to the students. This would further assist me in conducting a lesson appropriate to the audience. IT issues on the day of the teaching session highlighted the need to practice presentations in the environment in which they are to be delivered.

Creating the LFF taught me the importance of constructing clearly worded, unambiguous questions. The LFF teleconference demonstrated the benefit of collaborate discussions. Designing the LFF also enabled the consolidation of my own knowledge on survey design and analysis. Due to the broad experience of my LFF group, the teleconference discussion provided me with additional strategies which I could use to improve my analysis in the future.

Appendix 1: LFF

Lessons from the Field number 2

Survey design and analysis of multiple response questions

The LFF teleconference has been booked for Friday the 2nd of May, 2019 for 2:00PM EST.

The answers for the case study are due on Friday the 2nd of May.

Participants are to dial…….and then enter the pin number which is…….

If you have any difficulties connecting to the teleconference, you can contact Sophie on 0412 317 347 or at Sophia.Bowman-Derrick@dhhs.vic.gov.au

About this LFF

This LFF has two parts. Part 1 consists of some questions about survey design in relation to a real-life case study survey on seasonal influenza. Part 2 consists of a brief exercise using the package mrtab to analyse multiple response questions in STATA.

Learning objectives

By the end of this LFF you should be able to:

1. **Understand the steps in designing a questionnaire**
2. **Understand the potential pit-falls associated with questionnaire design**
3. **Have an understanding of how to construct and word a questionnaire**
4. **Be able to apply some of the techniques that are available for the analysis of multiple-response questions in STATA**

Part 1. Survey design

You are the new MAE at the Department of Health and Human Services (DHHS) in Victoria. The media and communications team has recently conducted a survey which intended to inform their upcoming flu season campaign. They had hoped to determine the potential reasons underlying low vaccination rates for influenza in the general population. The results of the survey were to be used to inform a media campaign to increase influenza vaccination rates. The media team asks for your help with analysing the survey results.

Unfortunately, the epidemiology team was not consulted as part of the planning and design of the survey. Consequently, there are some issues with the survey, making the analysis challenging. The survey participants were heavily biased as more than half of them identify as health-care workers (HCW). You are told that the survey was largely disseminated via the Better Health website. This survey will be conducted again during the next flu season.

Your role is to:

- **Identify how the survey can be improved for the following flu season**
- **To determine how to analyse survey questions where a respondent can select multiple answers**

The following readings (provided with this document) may be useful for part 1 of the LFF:

- *Surveys and sampling design.* (*Chapter 6 Field Epidemiology).* Page 1-7 only are relevant to survey design
- *Hands-on guide to questionnaire research. Selecting, designing and developing your questionnaire.* Page 1312-1315.
- *Developing a Questionnaire.*
- *Tip Sheet on question Wording.*

To get started, open the pdf file titled “Seasonal influenza survey.”

Question 1. Based on the survey questions provided, what are three possible hypotheses that this survey aimed to address?

1. *That people lacking knowledge of influenza have low rates of influenza vaccination*
2. *That people who have regular contact with health professionals are more likely to be vaccinated for influenza.*
3. *Those who have had influenza in the past are more likely to be vaccinated for influenza.*

Question 3. What are three ways in which the flow of the survey be improved?

1. *With the use of skip patterns (unclear if these were utilised in the original survey design)*
2. *Using introductory statements to explain why certain questions are being asked. E.g. Question 1. To question 3. The following questions aim to assess your knowledge of*

Question 4. List two questions from the survey that you think are not relevant to the objective of understanding the reasons underlying low vaccination rates for influenza. Briefly explain why you think the question is not relevant.

1. *"Were you tested by a doctor?" This question really relates to diagnostic practices of doctors and isn't relevant to understanding low vaccination rates.*
2. *"If you went to hospital when ill with the flu, what were your main reasons for going to hospital?" This question relates to understanding reasons for hospital attendance, but was possibly included to assess perceived severity of influenza.*

Question 5. For the following three questions from the survey, how can the wording or structure of the question be improved?

1. **"Are you employed in any of the following sectors? (Tick all that apply)"**
2. **"How much influence does advice from a health professional have on your decision to be immunised, or encourage others to be immunised?"**
3. **"Have you ever had flu?"**

1. *The question could ask which category best describes the main area of the respondent's employment. The question could be more specific about the employment categories e.g. Health = those working in direct contact with patients, for example nurses, doctors, pharmacists*
2. *This question could be broken down into several questions, for example, a series of statements asking the respondent to rate the degree to which the situation influences their decision to be immunised. E.g. Your GP recommends the influenza vaccine. Please select the answer which most accurately represents how strongly this influences your decision to be vaccinated: a) not at all, b) somewhat, c) strongly*
3. *This question could be improved by better defining what is meant by flu. E.g. Have you ever had a flu or flu-like illness where you had chills, fever, fatigue, a dry cough? I.e. define the difference between cold and flu for the respondent.*

Question 6. "What was your reason for not getting the flu vaccine? (Tick all that apply)".

Suggest how this question could be changed to better address the objective "understanding the reasons underlying low influenza vaccination rates". E, g, could additional answer choices be provided, or could the answer choices be given in a different way?

The respondent could have been asked to rate a series of statements, and how strongly they agree or disagree with each statement. For example for the statement "I am fit and healthy, so I'm unlikely to get the flu", the respondent could have been asked their level of agreement from strongly disagree, disagree, neutral, agree to strongly agree.

Question 6. What are three steps that should be completed before beginning to write the questions for a survey?

1. *Literature search to ensure the question hasn't already been answered*
2. *Literature search to determine if a survey is the best way to answer this question*
3. *Identify your objective/research question in order to focus your questionnaire*

Question 7. Half of the respondents were health care workers. How could the survey have been better disseminated to get a representative sample of the population?

1. *Social media advertising*
2. *Mail out*
3. *Not exclusively disseminating the survey via the Better Health website – using other websites/networks as well – e.g. doctor's office, chemist, supermarket/other retail/random text message numbers/email of past disease notifications?*

Part 2. Analysing multiple response questions in STATA

The following documents may be useful in answering the questions in this section:

1. *Tabulation of multiple responses*
2. *STATA FAQ Dealing with multiple responses.*

You only have one day left to analyse the Seasonal Influenza survey results, as the media team need to get their campaign underway. There are a few tricky questions where the survey has allowed more than one answer, and you are not sure how to perform the analysis for these. Luckily, you discover that a STATA package has been written to conduct this analysis efficiently.

You need to do a bit of background reading to understand the management of variables for multiple response questions first. Please answer the following questions.

Question 1. What are the two modes that data may be presented in for questions allowing respondents to provide more than one answer? What is the difference between these two modes?

Indicator mode (or dummy variables). Each possible answer is coded as 0 or 1, with 0 indicating that the item was not marked, and 1 indicated the item was marked on the survey.

Polytomous mode. There is one variable for each possible answer. In this mode, each data point is represented by a first, second, third response etc. Responses may be

Question 2. Please identify the mode of the variables for the following 2 tables (fill in the blank).

Question: Are you employed in any of the following sectors?

Variables: Education, Health, Aged care, Government, Private/Corporate sector, I do not work in any of these sectors

Mode: _____

Table 1.

Respondent id	Education	Health	Aged Care	Government	Private/Corporate sector	I do not work in any of these sectors
1	0	1	1	0	1	0
2	1	0	0	1	1	0
3	1	0	0	1	0	0
4	0	0	0	0	1	0
5	0	0	0	0	1	0
6	0	1	1	1	0	0

Question: Are you employed in any of the following sectors?

Variables: 1: Education, 2: Health, 3: Aged care, 4: Government, 5: Private/Corporate sector

Mode: _____

Table 2.

Respondent id	Employment 1.	Employment 2.	Employment 3.	Employment 4.	Employment 5.
1	0	2	1	0	5
2	1	0	0	5	4
3	4	0	0	3	0
4	0	0	0	0	2
5	0	0	0	0	5
6	0	5	3	4	0

Question 3. In table 1 (above), what area/s was respondent 1 employed in?

Respondent no. 1 answered that they do work in Health, Aged Care and in the Private/Corporate sector.

Question 4. In table 2 (above), what area/s was respondent 2 employed in?

Respondent 2 works in Education, Private/Corporate sector and Government

To analyse a question where respondents have provided multiple answers, you could manually create dummy variables, however this would be quite time-consuming. The package "mrtab" enables one-way tables of frequency distribution of responses, two-way tables and frequencies of the number of responses and respondents to be easily calculated.

Question 5. Which mode will the package mrtab assume data is in?

Indicator mode

Fortunately, you have already completing cleaning of the survey dataset. Follow the instructions below to finish your analysis of the multiple-response questions:

Open STATA and import the Excel file titled "Seasonal influenza survey data"

Install the package mrtab by typing:

```
ssc install mrtab
```

The first data sheet is in indicator mode. Type in the following code:

```
mrtab Washingmyhandsproperlyafter- Idontknow
```

Question 6. What percentage of respondents answered "Disposing of tissues into the rubbish?"

97.76%

Create a graph of the results obtained using the mrtab function, using the command mrgraph. Type in the following code. If performing an analysis, you would need to rename the variables so that they fit onto the graph

```
Mr graph bar
```

Now import the second Excel sheet entitled "polytomous variables." Type in the following code:

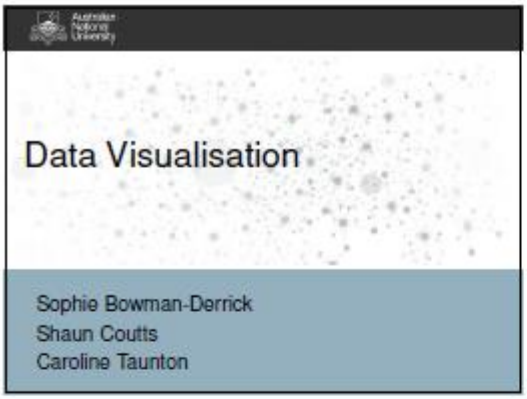
```
Mrtab ReasonA-Reason8, poly
```

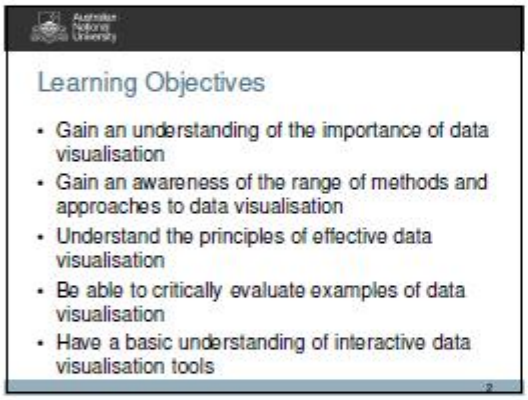
Question 7. What percentage of respondents selected "The vaccine is free for me"? Please refer to the data dictionary on the third tab in the Excel spread sheet provided

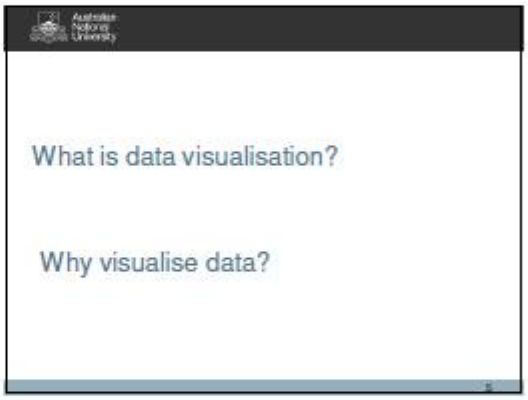
Reason 4 =44.9% of cases

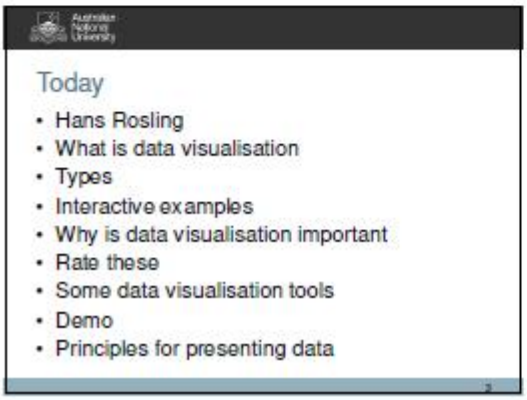
Congratulations! The media team were so impressed with your analysis that they have asked you to oversee designing the survey for next year!

Appendix 2: First year MAE teaching session



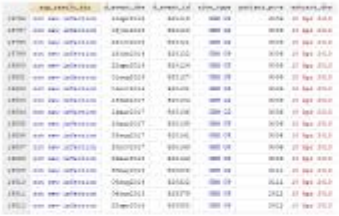






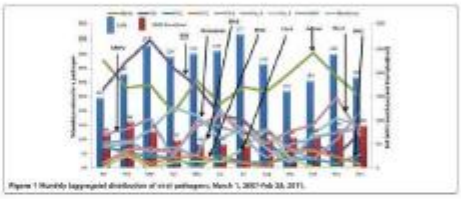
Types of data visualisation

Line listed data



Types of data visualisation

Standard graphs

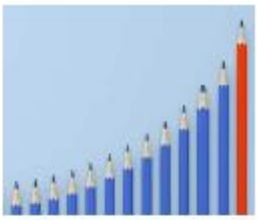


Types of data visualisation

Summary tables

Types of data visualisation

Themed graphs

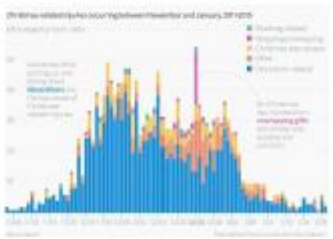


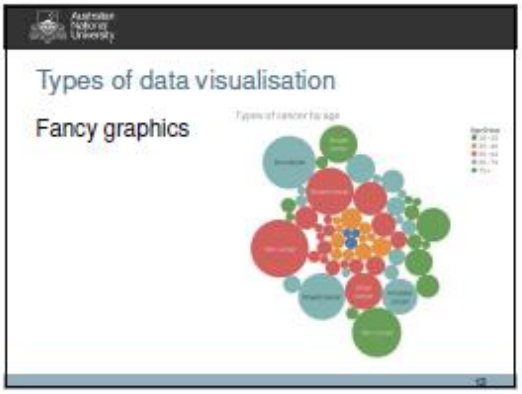
Types of data visualisation

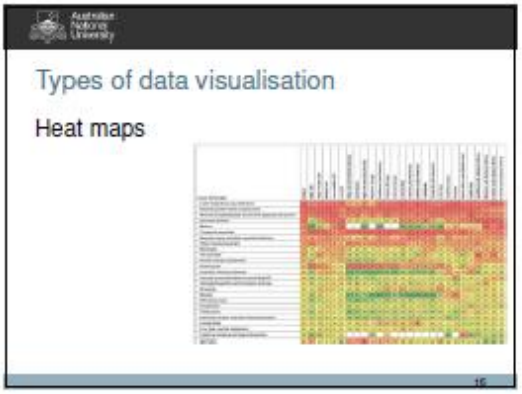
Data tables

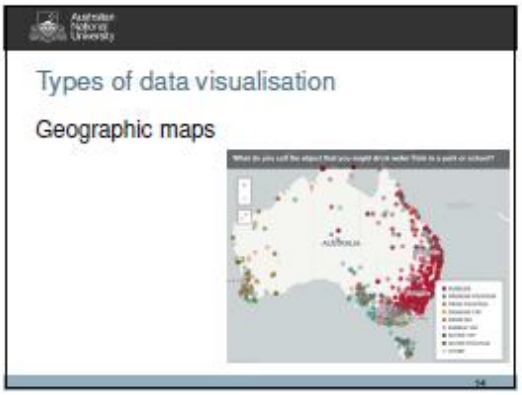
Types of data visualisation

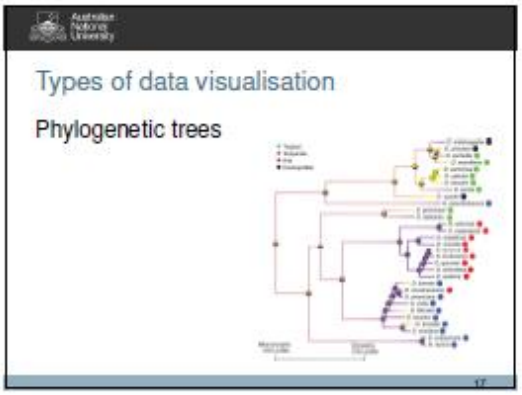
Epi curves

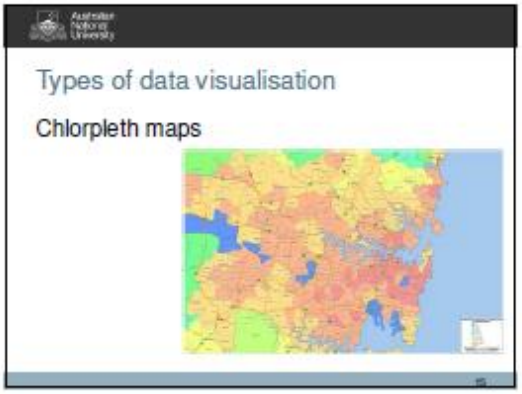


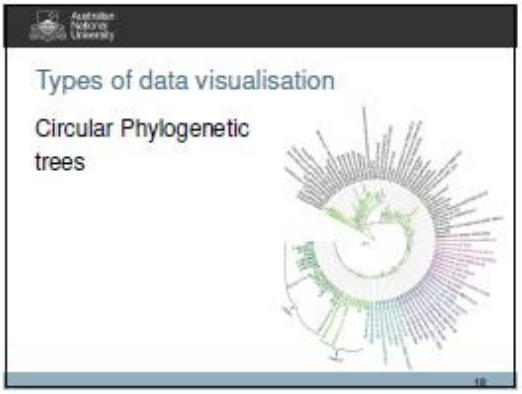

Types of data visualisation
Fancy graphics

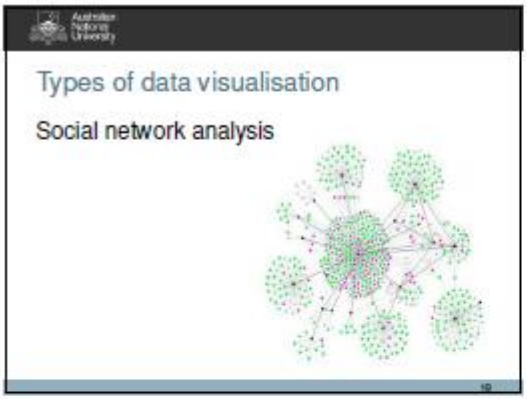

Types of data visualisation
Heat maps

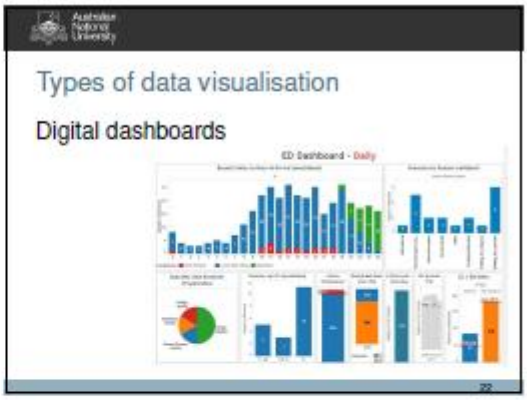

Types of data visualisation
Geographic maps

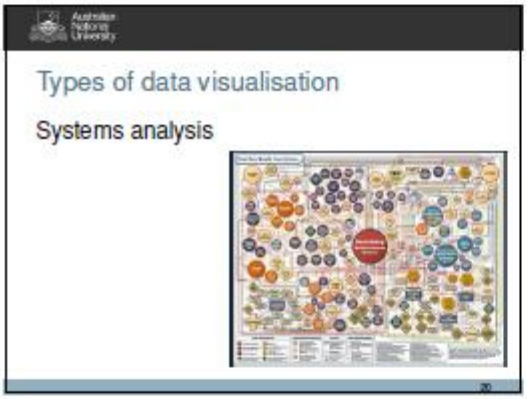

Types of data visualisation
Phylogenetic trees

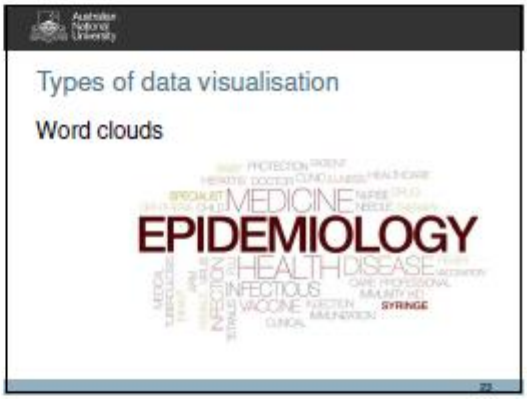

Types of data visualisation
Chlorpleth maps

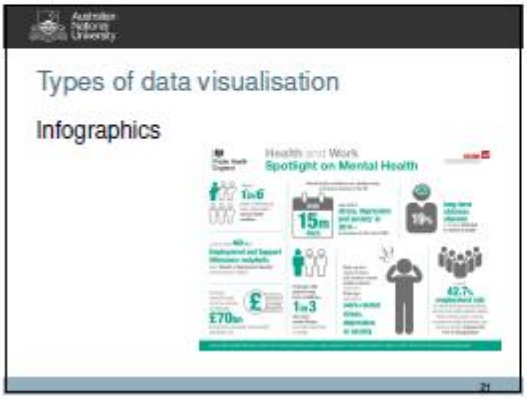

Types of data visualisation
Circular Phylogenetic trees

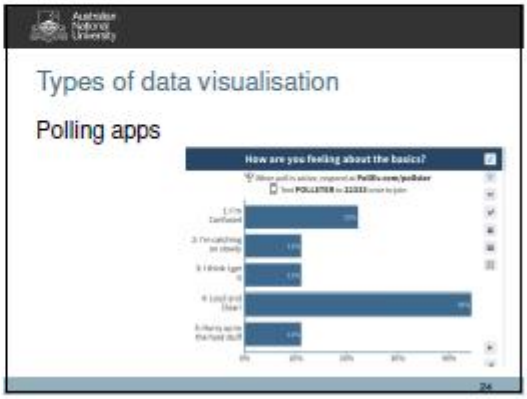

Types of data visualisation
Social network analysis


Types of data visualisation
Digital dashboards


Types of data visualisation
Systems analysis

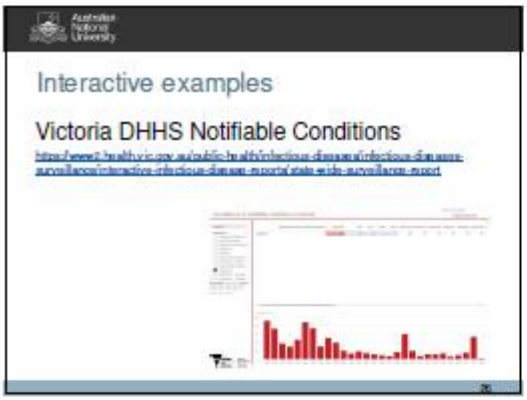

Types of data visualisation
Word clouds
EPIDEMIOLOGY
MEDICINE
HEALTH
DISEASE
INFECTIOUS
VACCINE
SYRINGE


Types of data visualisation
Infographics
Health and Work
Spotlight on Mental Health


Types of data visualisation
Polling apps
How are you feeling about the basics?

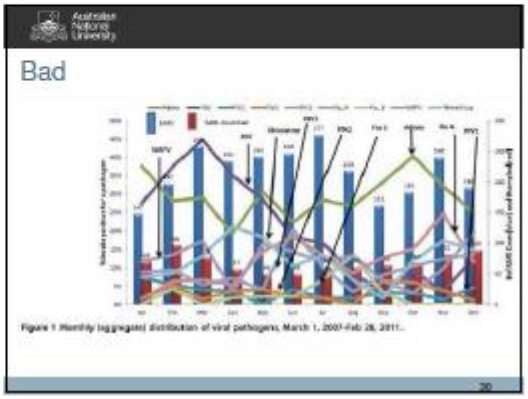

Interactive examples
Institute for Health Metrics and Evaluation


Interactive examples
Fitbit


Interactive examples
Victoria DHHS Notifiable Conditions


Good


Interactive examples
Gapminder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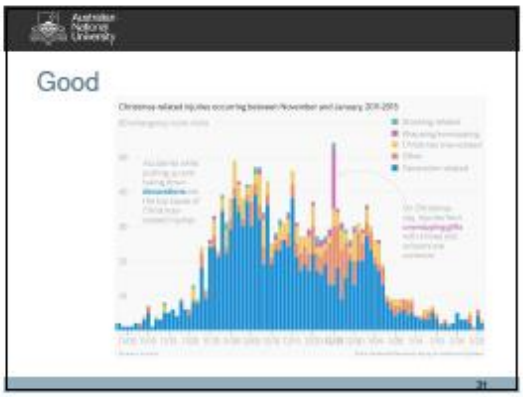
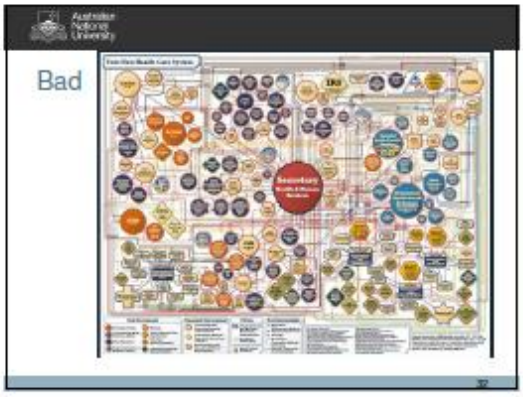

Good


What do you think?
Yoga as a Complementary Health Approach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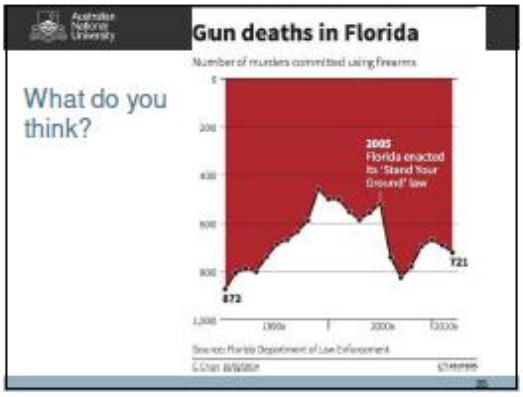
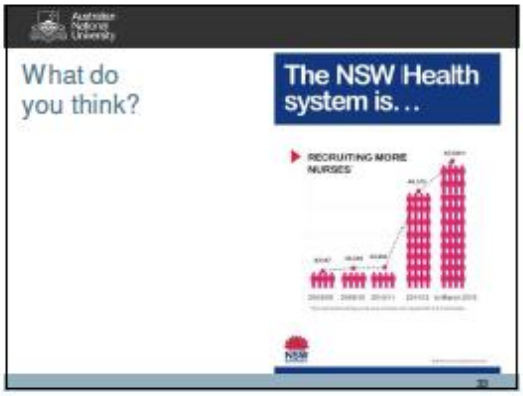
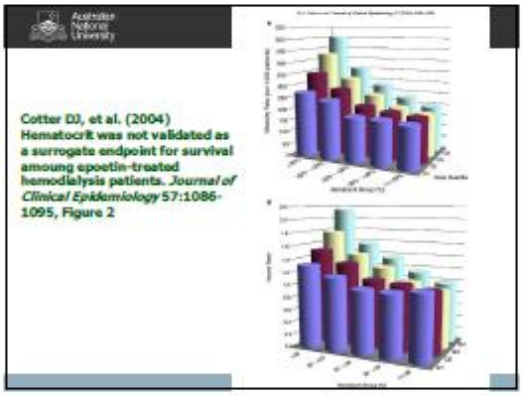
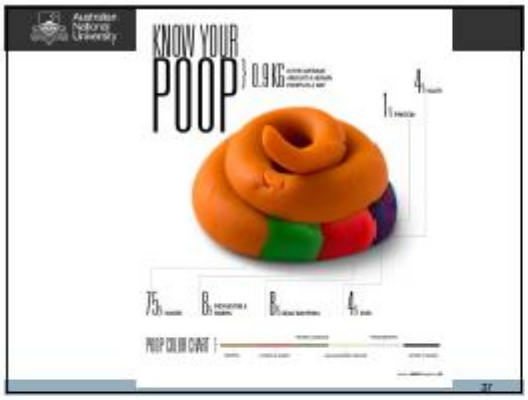

What do you think?
Gun deaths in Florida
2005
Florida enacted its 'Stand Your Ground' law
872
721


What do you think?
The NSW Health system is. . .
RECRUITING MORE NURSES

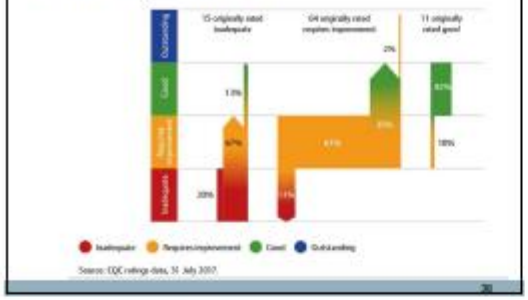

Cotter DJ, et al. (2004) Hematocrit was not validated as a surrogate endpoint for survival amoung epoetin-treated hemodialysis patients. Journal of Clinical Epidemiology 57:1086-1095, Figure 2



Interactive data visualisation tools

- Hundreds of products on the market
- Very few are free
- Be very mindful of data uploaded
- Consider where data are hosted and data regulations (or lack of them) in different countries

What do you think?

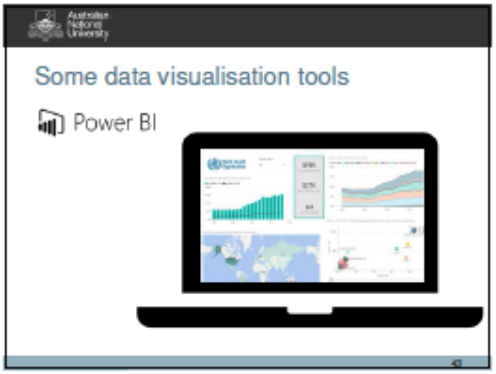


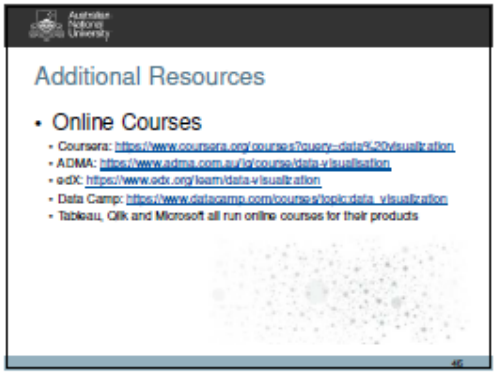
Some data visualisation tools

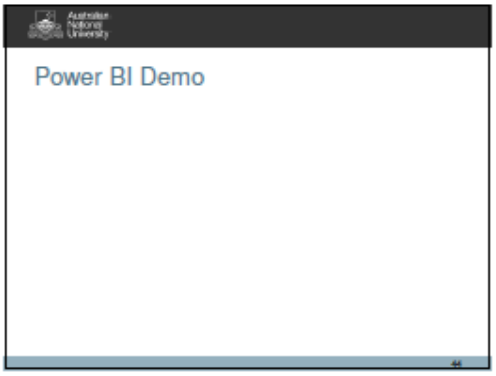
Why visualise data?

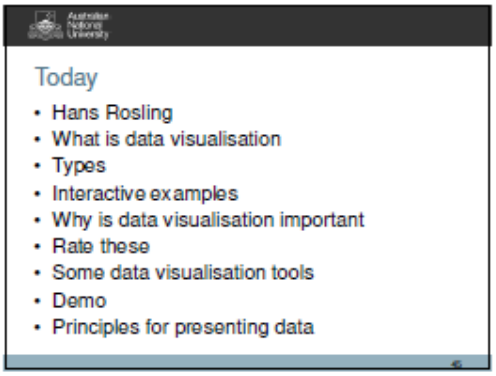
- To Communicate data
- To identify trends e.g. geographic distribution, phylogenetic relationships
- To enhance detection of patterns
- To Identify outliers

Some data visualisation tools


Some data visualisation tools
Power BI


Additional Resources
Online Courses
Coursera: https://www.coursera.org/courses?query=data%20visualization
ADMA: https://www.adma.com.au/lg/course/data-visualisation
edX: https://www.edx.org/learn/data-visualization
Data Camp: https://www.datacamp.com/courses/topic/data_visualization
Tableau, Qlik and Microsoft all run online courses for their products


Power BI Demo


Today
Hans Rosling
What is data visualisation
Types
Interactive examples
Why is data visualisation important
Rate these
Some data visualisation tools
Demo
Principles for presenting data